Ukraine Biological Threat Reduction Program (BTRP)
Cooperative Biological Research (CBR) Project

Regional field-to-table risk assessment of the spread of African swine fever virus (ASFV) across Ukraine in wild fauna and via consumer trade routes – insight into the development of effective ASFV quarantine strategies and public policy

UP-10 PROJECT FINAL REPORT
for the period 08 October 2019 – 30 June 2020

Prepared for:



Prepared by:

BLACK & VEATCH SPECIAL PROJECTS CORP.



in collaboration with:





22 July 2020

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1. Project Information

1.1. Task Order 04 Information

1.1.1. Contract Number

HDTRA1-08-D-0007-0004

1.1.2. Project Title

Biological Threat Reduction Program (BTRP)

Cooperative Biological Engagement Program (CBEP)

Phase IIb in Ukraine – HDTRA1-08-D-0007-0004

1.2. CBR Information

1.2.1. Project number: UP-10

1.2.2. Project title: Regional field-to-table risk assessment of the spread of African swine fever virus (ASFV) across Ukraine in wild fauna and via consumer trade routes – insight into the development of effective ASFV quarantine strategies and public policy

1.2.3. Performance Period

Project Period of Performance (PoP) – 08 January 2019 – 30 June 2020 (including no-cost extension [NCE])

Reporting PoP – 08 January 2019 – 30 June 2020

1.2.4. Performing Organization

Black & Veatch Special Projects Corp. (BVSPC)

1.2.5. Teaming Partner

Metabiota, Inc.

Labyrinth Global Health, Inc.

1.3. Threat Reduction

1.3.1. Project Impacts

- Provided validated proof that ASFV-infected meat is circulating within illegal trade networks within Ukraine in sufficient quantities to be detected through random surveys (manuscript in preparation).
- Increased understanding of the risk posed by ASFV in Ukraine and for communicating and applying those risk models to neighbors and international partners.

- Identified potential sources of risk for transboundary spread of ASFV, either into or from Ukraine to the European Union (EU) and other international trade partners.
- Identified gaps that, once addressed, will increase the ability of the regions within Ukraine's biosurveillance network to respond to, and prevent the spread of, future transboundary disease threats.
- Validated the need for renewed public outreach and information campaigns designed to educate the public of the risk posed by ASF, and other diseases of concern, and ways to avoid contributing to disease transmission.
- Project methods and approaches, specifically the fundamentals of conducting and interpreting KAP Surveys, were directly integrated into new college education curricula by the National University of Life and Environmental Sciences (NULES).
- Established interagency collaborations, government-private sector relationships, and network of SMEs from different countries that, if maintained, could enhance the detection of, and response to, future biological threats.

1.3.2. Future Recommendations

- **Develop backyard farm interventions along major transportation and trade corridors.** Based on feedback received from UP-10 participants at the State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP), farmers are resistant to preventive and control measures pertinent to home slaughtering, culling, and the timeline for quarantine. Coupling this stance with the impact that anthropogenic factors have on ASFV transmission, expanded behavioral assessment is required to ascertain key risk factors (e.g., regulations that are disregarded by farmers) and subsequently determine measures (e.g., biosecurity) that would compel increased adoption and adherence to ASF control policies. Ultimately, additional insight into the socio-economic and behavioral drivers that exacerbate the spread of ASF is needed. In this manner, gaps can be identified and addressed. To make this effective, communication via targeted and succinct messaging is needed, emphasizing concern of the disease and raising awareness of safety practices. Such efforts would support behavioral adaptation to, and acceptance of, measures required to mitigate the endemic potential of ASFV.

- **Identify challenges imposed by ASF control policies and develop recommendations for modifications that would support increased compliance.** The feasibility of current ASF policies needs to be evaluated, with consideration given to the constraints experienced by those who must abide by such policies (e.g., farmers, sellers, buyers). Policy adjustments will be needed to reasonably achieve acceptance (e.g., revise quarantine regulations at time of purchase so that the responsibility is placed on the buyer vs the

seller, as the former holds greater risk of spreading ASF to their herd through the introduction of the new animal).

- **Create the framework by which to transition unlicensed community markets, "gray markets", into a more regulated dynamic.** Such markets, which are culturally accepted, are not compliant with ASF policy prohibiting the sale of meat from unapproved/unofficial sales points. By creating another tier of legitimate market (e.g., a "Farmers Market" that has a set time, location, minimally acceptable biosecurity measures, and is reasonably monitored), regulators may achieve greater compliance. That is, new/adjusted policies could provide opportunity for limited licensing, such as limited for rural sales. In this manner, a level of control and oversight, though less than ideal, can be attained.

- **Increase the ability to interpret findings and identify actionable results that contribute to ASF risk mitigation.** As presented in Section 2.6, the UP-10 project team identified ASFV-contaminated meat products purchased from illegal vendors. These efforts represented a qualitative, not quantitative, survey, and though the number of positive samples was limited, these findings raise concern of ASFV transmission via the illegal trade network. However, the National authorities have applied a quantitative interpretation, promoting the inaccurate perspective that limited positive results indicate that little to no contaminated products are publicly sold. Thus, enhanced understanding and acceptance of the relevance of the study's qualitative results is imperative. Through such efforts, gaps in the biosurveillance network that permit the spread of disease, via illegal sales and other anthropogenic factors, can be identified and overcome.

- **Develop and implement activities through ASF policy adjustments that instill trust and understanding between regulators and those required to abide by the mandated policies (e.g., farmers, buyers, sellers).** To achieve consensus on the need to mitigate ASF, mutually beneficial approaches are required to adjust perceptions of all those involved. Regulation changes should promote cooperation and disincentivize illegal activity. For example, rather than enforce fine-based policies, regulations should support incentives for compliant behavior (e.g., provide incentives to individuals who report ASF outbreaks). Additionally, the dialogue on ASF risk mitigation should be inclusive of both stakeholders developing and implementing policies and those who must abide by such policies. In this manner, mutual interests can be met across the range of stakeholders (e.g., regulators, famers, sellers, etc.) to enhance support acceptance of existing, adapted, or new regulations.

- **Re-evaluate the existing gaps in the biosurveillance network, including the lack of authority to directly address illegal sales and other practices that contribute to the anthropogenic spread of ASFV and other pathogens of concern.** A significant contributor to the existence of this gap in the biosurveillance network was the dissolution of the Veterinary Police in 2016. While SSUFSCP is supposed to officially ensure food safety in the country, they currently do not have legal powers to fine or ban individuals for illegal sales. As of today, this power belongs exclusively to the National Police of Ukraine. As such, it behooves policymakers and senior decisionmakers to develop and implement legislative and regulatory changes to fully fund and empower SSUFSCP to both conduct food safety surveillance and enforcement.

- **In summary, by examining social and economic motivations of the varied stakeholders and attempting to more comprehensively understand perceptions, policies could be developed that will reduce the spread of ASFV and possibly even eradicate the disease in some regions.** Importantly, existing, adjusted, and new policies will be accepted if viewed as beneficial to everyone involved. Taking this into consideration, policy reform must include realistic and timely compensation for lost production, specifically euthanized pigs. For example, if farmers knew that they would receive market value for a pig killed due to ASFV, then they would not need to sell a sick pig quickly before the disease was detected/identified.

2. UP-10

2.1. Project Description

Ukraine UP-10 Quarterly Factsheet Information

"Regional field-to-table risk assessment of the spread of African swine fever virus (ASFV) across Ukraine in wild fauna and via consumer trade routes – insight into the development of effective ASFV quarantine strategies and public policy"

2.2. Research Objectives

2.2.1. Problem Description

ASFV is a highly infectious agent that causes a devastating and frequently fatal disease, African swine fever (ASF), in domestic pigs and wild boar. Currently, there are no effective treatments or vaccines to offset the threat of ASFV, which is spreading rapidly through naive swine populations in Ukraine and neighboring countries. Despite intensive international efforts, vaccine development seems highly unlikely in the near future. The only available methods for disease control depend on strict quarantine and the slaughter of infected animals, as well as animals in close proximity to those infected (1). Disease outbreaks often inflict significant economic loss due to the widespread culling of affected animals, production losses, and implementation of trade restrictions to prevent further

viral spread within the region and across regional borders. As a potential transboundary disease capable of severe economic damage, ASF is a significant concern within the European Union (EU) and neighboring countries, including Ukraine (2). The identification and confirmation of the introduction of ASFV into Belgium on 14 September 2018, a location approximately 1,000 km from the nearest ongoing outbreak, caused great concern throughout the EU, with one published statement going so far as to say "This new outbreak may represent a new change in the epidemiologic situation of ASF worldwide, suggesting that the disease may have reached pandemic proportions" (3-9).

In Ukraine and neighboring countries, anthropogenic factors (human behavioral) and poor biosecurity measures most likely represent the biggest contributors to the rapid spread of ASFV. Supporting this hypothesis is the observation that transmission has been associated with major transportation and trade corridors from the North to the South and the East to the West within the territory of the country. As such, it is proposed that socio-economic drivers contribute to the maintenance and spread of ASFV after introduction into the rural agricultural network. Furthermore, potential lack of transparency in reporting outbreaks and poor biosecurity measures at rural holdings further increase the risk of virus spread through contaminated swill feed consumption by domestic swine and through the illegal disposal of carcasses in wooded and rural areas accessible to wild boar.

Due to socio-economic factors in rural areas of Ukraine, gray- and black-market trade represent a potentially significant source of transportation of ASFV-contaminated products within the country and across international boundaries. There is a significant gap in understanding the extent of contaminated products capable of serving as a reservoir of ASFV and facilitating the distribution of the virus within the region via both licensed community markets and non-licensed points of sale. These vulnerabilities highlight gaps in Ukraine's existing legislative and regulatory framework for controlling the spread of ASFV and other zoonotic diseases of concern in food and feed.

The severity of the epizootic situation is further reinforced following the report issued by the Food and Agriculture of the United Nations (FAO)/World Organization for Animal Health (OIE) Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) Standing Group of Experts on ASF in Europe on 29 May 2019, which stated that the Ukrainian delegation reported that ASF is now considered to be endemic in Ukraine (10). Due to the advanced stage of the spread of the virus through Ukraine, it is imperative that efforts be made to address gaps in public outreach and ensure that public education becomes a leading component in preventing further spread of ASFV and the disease becoming endemic nationally. Central to this must be raising awareness among farmers, veterinarians, and hunters regarding the biological

risk presented by ASF and the clinical signs of the disease, as well as ensuring awareness and tracking of animal movement. This will contribute to the economic sustainability and stability of agricultural markets within Ukraine.

2.2.2. Research Goals

This project assessed the relationship between hypothesized risk factors and their contribution to or impact on ASFV distribution/spread in Ukraine. To accomplish this, the following project Goals were pursued:

- **Goal 1:** Define geographical and environmental factors associated with establishment and spread of ASFV through wild boar movements
- **Goal 2:** Track anthropogenic and socio-economic factors
- **Goal 3:** Train, educate, and conduct outreach to inform public perception and Public Policy decisions

2.2.3. Expected Impacts

- Improved understanding of the mechanisms contributing to the distribution of ASFV within Ukraine using established data sets to connect geographical data and anthropogenic factors.
- Provided decisionmakers within the Ministry of Agrarian Policy of Ukraine recommendations for forecasting tools and evidence-based/data-driven control strategies.
- Improved public understanding of the risk posed by ASFV and how to implement effective local biosecurity measures against ASFV.
- Identified geographical circulation patterns of ASFV in Ukraine.
- Improved understanding of the general prevalence of ASFV in rural/ unregulated trade networks within targeted areas.
- Informed improvements to biosecurity control measures to limit ASFV spread within the country and across regional borders through enhanced understanding of risk factors associated with ASFV.
- Improved understanding of biosecurity practices and public outreach measures within the country and across regional borders through enhanced understanding of socio-economic factors and perceptions that contribute to the spread of ASFV.

2.2.4. Project Participants

- State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP), Kyiv, Ukraine
  Principal Investigator: Mr. Mykola Sonko, Chief of the Department of Animal Health and Welfare

- The State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE), Kyiv, Ukraine
  Principal Investigator: Dr. Andrii A. Mezhenskyi, Director

- National Scientific Center "Institute of the Experimental and Clinical Veterinary Medicine" (IECVM), Kharkiv, Ukraine
  Principal Investigator: Dr. Anton Gerilovych, Deputy Director

- Institute of Veterinary Medicine (IVM), Kyiv, Ukraine
  Principal Investigator: Dr. Serhii Nychyk, Director

- State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM), Kyiv, Ukraine
  Principal Investigator: Dr. Zinaiida Klestova, Deputy Director

- State Forest Resources Agency of Ukraine (SFRA), Kyiv, Ukraine
  Principal Investigator: Mr. Andrii Shelepylo

- National University of Life and Environmental Sciences (NULES)
  Principal Investigator: Dr. Volodymyr Polishchuk

- Kansas State University (KSU), USA
  Subject Matter Experts (SMEs): Dr. Stephen Higgs, Director, Biosecurity Research Institute; Dr. Dana Vanlandingham, Professor; Craig Beardsley, Principal Investigator

- SAFOSO AG, Switzerland
  SMEs: Dr. Marco De Nardi, Senior Consultant; Dr. Manon Schuppers, Director; Ms. Violeta Muñoz, resident of the European College of Veterinary in Public Health

- Labyrinth Global Health, Inc., USA
  SME: Dr. Karen Saylors, Principal Investigator

- Helmholtz Centre for Environmental Research – UFZ, Germany
  SME: Dr. Hans-Hermann Thulke

- University of Florida, USA
  SME: Dr. Jason Blackburn

2.3. Technical Approach

2.3.1. Methodology

Within the project, the following approaches were implemented in order to ensure comprehensive and efficient achievement of UP-10 key objectives.

- Spatial mapping and modeling technology (e.g., Geographic Information Systems-GIS) to analyze national data on host distribution and ASF notifications concerning wild boar.

- Spatially explicit risk factor study at the wildlife-livestock interface using existing data from forestry and swine industry.
- Modern qPCR techniques for ASFV identification in samples collected at non-licensed points of sale (e.g., pop-up markets) and from backyard holdings.
- Training, education, and outreach methods and approaches, including round-table discussions, concerning ASF.
- Capacity building for spatially explicit computational methodologies realized through a training fellowship for Ukrainian scientists facilitated by the University of Florida.

2.3.2. Description of Technical Approach

UP-10 expanded upon key technology and findings from previously completed BTRP-Ukraine projects UP-9, TAP-6, and TAP-4. BVSPC/Metabiota facilitated implementation in close coordination with the project's participants.

Ukrainian participation was coordinated in part by the SSUFSCP, which is responsible for establishing Ukraine's regulatory policy for ASFV, reporting on ASFV to the OIE, as well as overseeing the activities of SSRILDVSE. Academic partners from the NAAS and the Ministry of Education and Science of Ukraine (MESU) coordinated activities for socio-economic studies and surveys, as well as development of educational materials.

2.4. Schedule and Milestones

2.4.1. Schedule

Yellow reflects work that has initiated, green indicates ongoing work, gray represents the timeline for planned work, pink reflects partially completed work, and blue indicates completed work.

	Milestones and Tasks	Q1: 8 Jan – 7 Apr 2019	Q2: 8 Apr – 7 Jul 2019	Q3: 8 Jul – 7 Oct 2019	Q4: 8 Oct – 7 Jan 2020	Q5: 8 Jan – 7 Mar 2020	NCE 8 Mar- 30 Jun 2020	Comments
1.	**Define geographical and environmental factors associated with establishment and spread of ASFV through wild boar movements**							
1.1.	Perform spatial modeling of existing data on wild boar occurrence, habitat landscape structure, and seasonal movement across Ukraine.						**Completed**	Work conducted in coordination with CBR project UP-9 and the UP-10 Fellowship (see Section 2.6.1).
1.2.	Support capacity building for spatially explicit computational methodologies within the participating Ukrainian organizations.						**Completed**	Work conducted in coordination with CBR project UP-9 and the UP-10 Fellowship (see Section 2.6.1).
2.	**Track anthropogenic and socio-economic factors**							
2.1.	Ensure proper protocol and biosecurity throughout sample collection, shipping, and testing.						**Completed**	Of note, non-laboratory specialists and public sector participants quickly adopted proper BS&S practices during the purchase and sample packaging process.
2.2.	Analysis of biological samples of pork products from various stakeholders to test for ASF.						**Completed**	Work conducted suffered from not being fully differentiated from routine state surveillance.
2.3.	Demonstrate and document anthropogenic factors contributing to the spread of ASF in Ukraine and the need to implement effective biosecurity and control measures for preventing farm-to-farm and farm-to-wildlife spread.						**Completed**	UP-10 was the first study conducted in Ukraine and the region that demonstrated anthropogenic factors associated with activities outside of the commercial sector.

Milestones and Tasks		Q1: 8 Jan – 7 Apr 2019	Q2: 8 Apr – 7 Jul 2019	Q3: 8 Jul – 7 Oct 2019	Q4: 8 Oct – 7 Jan 2020	Q5: 8 Jan – 7 Mar 2020	NCE 8 Mar- 30 Jun 2020	Comments
2.4.	Assess the potential risk of ASFV spread within Ukraine and across regional borders via commercial trade routes of pigs and pig products, the illegal distribution and transport of pigs and pig products, and wild boar movements.						**Partially Completed**	While significant work was completed towards this Task, the objective was not fully realized due to COVID-19-related travel restrictions and cancellation of the 2020 BTRP Regional One Health Research Symposium.
3.	**Public policy and communication through training, education, and outreach.**							
3.1.	Establish a GIS and Computational short -term Modeling Fellowship.						**Completed**	Despite COVID-19-related travel disruptions, the Fellowship was implemented and successfully contributed to the graduation of two Train-the-Trainer (T3) trainers.
3.2.	Develop training curricula for GIS and perform outreach to inform local, regional, and national policy development.						**Completed**	GIS training continued despite COVID-19 impacts and also demonstrated the capacity of the two T3 trainers realized by the project.
3.3.	Develop audience-appropriate materials to support education and public outreach strategies.						**Completed**	UP-10 data were included into the official veterinarians' qualification advancement program; main target groups and key messages were identified during the workshop in Kyiv led by SAFOSO; KAP Survey tool included to the veterinarians' master program curricula at NULES.
3.4.	Educate and perform outreach to inform local, regional, and national policy development.						**Completed**	KAP survey results compelled high interest among Ukrainian officials; main target groups and key messages were identified during the workshop in Kyiv led by SAFOSO.

Milestones and Tasks		Q1: 8 Jan – 7 Apr 2019	Q2: 8 Apr – 7 Jul 2019	Q3: 8 Jul – 7 Oct 2019	Q4: 8 Oct – 7 Jan 2020	Q5: 8 Jan – 7 Mar 2020	NCE 8 Mar- 30 Jun 2020	Comments
3.5	Produce a minimum of two, Ukrainian-recipient led, peer-reviewed publications on this work.						Ongoing	Two manuscripts are undergoing final preparation, and others will be developed based on participant interest and availability of support from the UP-10 international collaborators and project's lead facilitators.

2.5. Project Presentations and Project Meetings

2.5.1. Presentations

- Oral and poster presentations at the DTRA Science Program Review, 17-20 September 2019, Warsaw, Poland (see Appendix I, item 1):
    - Poster (presented by Mykola Sonko, SSUFSCP): Sonko, M., Mezhenskyi, A., Sapachova, M., Sushko, M., Gerilovych, A., Solodiankin, O., Stegniy, B., Bezymennyi, M., Nychyk, S., De Nardi, M., Schuppers, M., Muñoz, V., Saylors, K., Beardsley, C. & Higgs, S. **Addressing the Human Contribution to the Spread of ASF in Ukraine: Implementing Tools to inform the Perception of the Public and Decision Makers to Help Mitigate Transboundary Disease Transmission**.
    - Poster (presented by Andrii Mezhenskyi, SSRILDVSE): Mezhenskyi, A., Sapachova, M., Sushko, M., Gerilovych A., Solodiankin, O., Stegniy, B., Kovalenko, G., Bezymennyi, M., Nychyk, S., Drown, D., Dubchak, I., Frant, M., Lange, C., Bortz, E., De Nardi, M., Schuppers, M., Saylors, K., Higgs, S. & Sonko, M. **Integration across BTRP-Funded ASF Mitigation Activities to Reduce the Threat of Transboundary Disease Transmission**.
    - Oral Presentation (presented by Andrii Mezhenskyi, SSRILDVSE): **Regional Field-to-Table Risk Assessment of the Spread of African Swine Fever Virus (ASFV) across Ukraine in Wild Fauna and via Consumer Trade Routes – Insight into the Development of Effective ASFV Quarantine Strategies and Public Policy**.

- Abstract submitted for 2020 ROHRS (this conference was suspended due to the COVID-19 pandemic: Polishchuk V., Sonko M., Solodiankin O., Rudova N., Gerilovych A., Nychyk S., Hudz N., Pavlenko A., Mustra D., Saylors K., Muñoz V., De Nardi M., Schuppers M. **The Cooperative Biological Research Project UP-10 as the Next Stage in Measures against ASF for Ukraine** (see Appendix I, item 2).

- Abstract submitted for Global African Swine Fever Research Alliance (GARA) Scientific Meeting, Kampala, Uganda; meeting was rescheduled to 25-27 August 2020 due to the COVID-19 pandemic (see Appendix I, item 3):

    - Andrii Mezhenskyi, Volodymyr Polishchuk, Serhii Nychyk, Anton Gerilovych, Andrii Pavlenko, David Mustra, Karen Saylors, Stephen Higgs, Mykola Sonko. **Investigating the Anthropogenic Contribution to the Spread of African Swine Fever virus (ASFV) in Ukraine through the Illegal Backyard and Non- Commercial Trade of Meat Products.**

    - Volodymyr Polishchuk, Mykola Sonko, Oleksii Solodiankin, Nataliia Rudova, Anton Gerilovych, Serhii Nychyk, Nataliia Hudz, Andrii Pavlenko, David Mustra, Karen Saylors, Violeta Muñoz, Marco De Nardi, Manon Schuppers. **UP10 – Building scientific evidence for improved ASF surveillance and control in Ukraine.**

2.5.2. Project Meetings

- **Kick-off-meeting (KOM)**. The KOM Meeting was comprised of two sessions conducted over a two-week period, 25-27 March 2019 (Session 1) and 02 – 04 April 2019 (Session 2) and included representatives from each of the Ukrainian Participating Institutes as well as SMEs.

    - Knowledge, Attitudes and Practices (KAP) survey (Task 3.4.2). During this meeting the target groups for the KAP survey were identified.

- **First Policy Working Group Meeting** (Task 3.4.3.a-b). This initial meeting took place on 21 May 2019 during the 4th Annual Regional One Health Research Symposium (ROHRS) in Kyiv, Ukraine. The meeting was called "Regulatory and Policy approaches for responding to ASF and Veterinary Transboundary diseases".

- **Outreach Working Group Meeting** (Task 3.4.1c). The Outreach working group met on 12-13 June 2019 in Kyiv, Ukraine. On this occasion, the target groups and the outreach strategy were agreed upon with stakeholders.

- **Workshop on the results and implications of the KAP survey** (Task 3.4.2.g). This workshop took place on the 17-19 December 2019 in Kyiv, Ukraine. Preliminary results on the KAP survey were presented and discussed.

- **First training on Geographic Information System (GIS)** (Task 3.2.b). This training, entitled "Introduction to GIS environment and spatial-temporal analysis" was conducted in Kyiv, Ukraine on 25-27 February 2020.

- **Second training on Geographic Information System (GIS)** (Task 3.2.c). Due to the COVID 19 pandemic, this training, entitled "The GIS Environment: how to make the best use of maps" was remodeled and implemented online on 01 July 2020.

- **Biological Safety and Biological Security (BS&S) Workshop** (Task 2.1c & Task 3.4.3). This workshop, entitled "Consumer Trade Routes and Food Safety: Identifying and Reducing Risks for the Spread of Veterinary and other Food-borne Diseases of Concern, with Particular Emphasis on ASF," was implemented in Kyiv on 17-21 February 2020.

- **Project Close-Out Workshop Series**:

    - **UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences** (19 June 2020), Microsoft Teams Platform Kyiv, Ukraine.

    - **UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences** (23 June 2020), Microsoft Teams Platform Kyiv, Ukraine.

      UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences (25 June 2020), Microsoft Teams Platform Kyiv, Ukraine.

    - **UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences** (30 June 2020), Microsoft Teams Platform Kyiv, Ukraine.

2.6. Technical Report

2.6.1. Findings Against Planned Objectives

GOAL 1. Define geographical and environmental factors associated with establishment and spread of ASFV through wild boar movements

Results and Discussion on Tasks 1.1 and 1.2:

- Task 1.1. Perform spatial modeling of existing data on wild boar occurrence, habitat landscape structure, and seasonal movement across Ukraine.
- Task 1.2. Support capacity building for spatially explicit computational methodologies within the participating Ukrainian organizations.

Results:
The following presents a summary of findings previously reported in Q4, coupled with new insights ascertained through subsequent training and the project's GIS and Modeling Fellowship, which collectively contributed to Goal 1 achievements.

- In support of Task 1.1.a, historical data on the density of pig farming in Ukraine were collected (**Table 1**). Official data are updated each year in mid-summer. Due to the COVID-19 pandemic, provision of updated information for 2020 continues to be delayed.

Table 1. Number of industrial pig farms and quantity of pigs according to the most recent official statistics.

Oblast	Number of Industrial Pig Farms	Number of Pigs per Farm
Vinnytsia Oblast	165	94,875
Volyn Oblast	71	65,041
Dnipropetrovsk Oblast	115	243,169
Donetsk Oblast	39	432,638
Zhytomyr Oblast	40	44,479
Zakarpattia Oblast	26	24,374
Zaporizhzhia Oblast	96	141,163
Ivano-Frankivsk Oblast	40	222,812
Kyiv Oblast	145	451,087
Kirovohrad Oblast	121	129,717
Luhansk Oblast	49	27,146
Lviv Oblast	140	195,160
Mykolaiv Oblast	63	38,972
Odesa Oblast	66	66,344

Oblast	Number of Industrial Pig Farms	Number of Pigs per Farm
Poltava Oblast	92	235,238
Rivne Oblast	56	33,184
Sumy Oblast	91	54,999
Ternopil Oblast	139	128,300
Kharkiv Oblast	62	122,897
Kherson Oblast	52	67,334
Khmelnytskyi Oblast	133	172,474
Cherkasy Oblast	122	191,817
Chernivtsi Oblast	76	61,449
Chernihiv Oblast	81	120,602
Total	**2,080**	**3,365,271**

- In support of Task 1.1.c, data on wild boar populations and geographic locations were collected (**Table 2** and **Figure 1**).

Table 2. Information on the number of wild boar according to official statistics.

Oblast	Number of Wild Boar in 2019 (as of 01 January 2020)	Number of Hunted Wild Boar in 2019 (as of 20 December 2019)	Number of Wild Boar Found Dead for Various Reasons
Vinnytsia Oblast	1410	176	0
Volyn Oblast	1423	184	10
Donetsk Oblast	573	19	13
Dnipropetrovsk Oblast	599	18	1
Zhytomyr Oblast	2985	196	5
Zakarpattia Oblast	2397	199	0
Zaporizhzhia Oblast	628	12	77
Ivano-Frankivsk Oblast	1665	123	11
Kyiv Oblast	578	20	0
Kirovohrad Oblast	473	31	1
Luhansk Oblast	206	2	2
Lviv Oblast	3980	285	4
Mykolaiv Oblast	765	42	0
Odesa Oblast	831	27	2
Poltava Oblast	1177	67	0
Rivne Oblast	523	41	0
Sumy Oblast	675	38	0
Ternopil Oblast	930	70	0
Kharkiv Oblast	761	9	0
Kherson Oblast	334	0	6
Khmelnytskyi Oblast	1090	61	0

Oblast	Number of Wild Boar in 2019 (as of 01 January 2020)	Number of Hunted Wild Boar in 2019 (as of 20 December 2019)	Number of Wild Boar Found Dead for Various Reasons
Cherkasy Oblast	1167	60	0
Chernivtsi Oblast	1181	111	48
Chernihiv Oblast	1492	52	0
Total	**27 843**	**1 843**	**180**

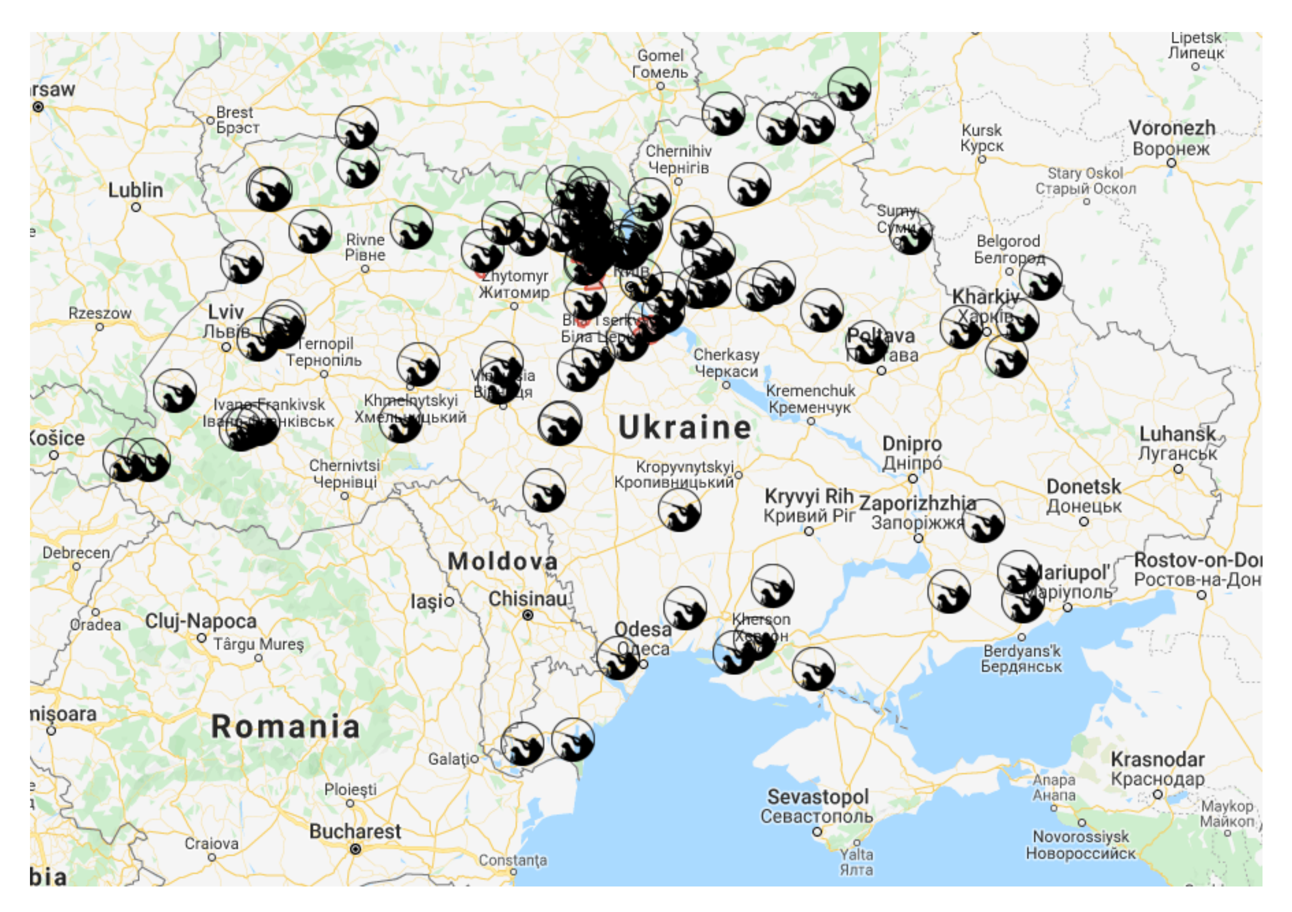


Figure 1. Distribution of hunting farms affiliated with the Public Organization Ukrainian Society of Hunters and Fishermen.

- In support of Task 1.1.e., and with the oversight of UP-10 participant Volodymyr Polishchuk (NULES), project team members continued to collect information on ASF outbreaks in Ukraine through various sources; e.g., http://www.asf.vet.ua/index.php/asfinukraine. Such efforts contributed to mapping ASF outbreaks identified as of 31 December 2019 (**Figure 2**).

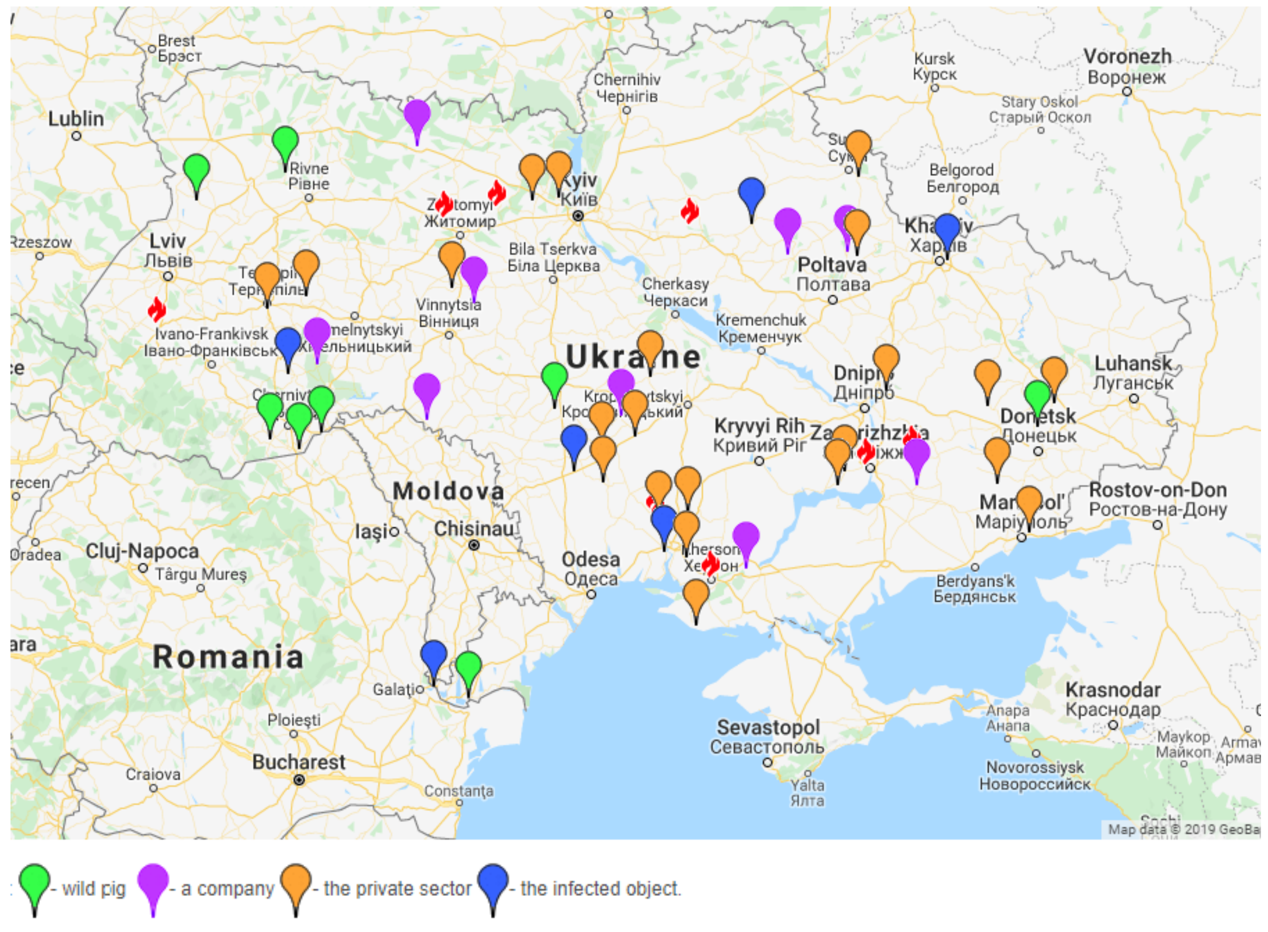


Figure 2. Map of ASF outbreaks as of 31 December 2019.

Discussion:

The project team pursued activities aimed at enhancing the capacity to understand and perform methodologies for spatial modeling. In addition to the aforementioned information collected and conveyed by NULES (**Figures 1-2** and **Tables 1-2**), two GIS training events were conducted. The first provided an introduction on spatial-temporal analysis; whereas, the second focused on the utility/application of maps. Through these efforts, new insights were acquired, which will contribute to the expansion of datasets and have equipped team members with the knowledge necessary to participate in future, more advanced, training (e.g., in remote sending methods for environmental monitoring and statistical analyses).

UP-10 also provided the unique and highly valuable opportunity for launching the first Ukraine CBR-related fellowship (Task 1.2.d.), which focused on GIS and modeling techniques. A competitive process was employed to select an individual for participation in this project-based initiative implemented in coordination with the University of Florida (Gainesville, FL). Following a formal

call for applications, UP-10 participant Maksym Bezymennyi was selected as the Fellow, and during a two-month tenure at the University, he participated in training and mentorship sessions focused on a number of spatial analysis techniques and mapping approaches. Topics included, but were not limited to the following activities:

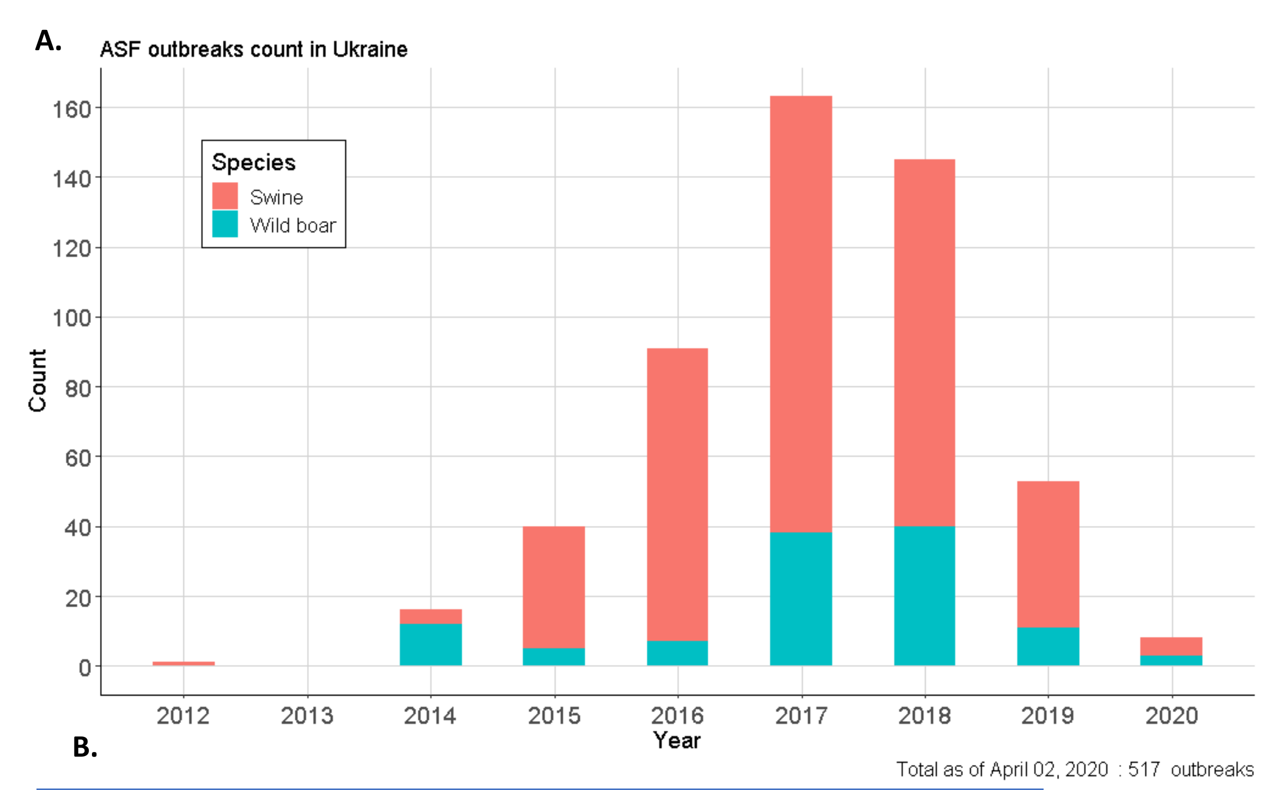
- Creating maps in R using the packages fp, sf, tmap, ggplot2, raster, and others, with focus on a range of tasks, such as data preparation, handling spatial data, creating spatial objects, checking coordinate systems, re-projecting spatial data, reading/writing shape files, and visualizing maps and charts. A sampling of figures generated as a result of these efforts is presented in **Figures 3-6.**
- Calculating descriptive spatial statistics in R using the aspace package: Mean center, standard distance, and standard deviational ellipse (**Figure 7**).
- Performing trend surface analysis (global polynomial interpolation) using the Geostatistical Analyst toolset in ESRI ArcMap software (**Figure 8**).
- Conducting point pattern analysis in R using the spatstat package: Multi-distance spatial cluster analysis (K-function, bivariate K-function), average nearest neighbor analysis, as well as kernel density estimation (KDE) and dual-kernel density estimation (**Figure 9**).

Importantly, the Fellowship allowed for highly relevant linkage to CBR Project UP-9, “The spread of African swine fever virus (ASFV) in domestic pigs and wild boar in Ukraine-Building capacity for insight into the transmission of ASFV through characterization of virus isolates by genome sequencing and phylogenetic analysis”. With shared interest in the epidemiology of ASFV transmission, both UP-9 and UP-10 project teams mutually benefited from the insights ascertained through this initiative. As conveyed via the in-depth analysis described in the UP-9 Option Year 1 (OY1) Final Report, spatio-temporal mapping and modeling of ASF outbreaks have highlighted the role of wild boar in the spread and persistence of the disease, as well as epidemic clustering, transboundary threats, and seasonality.

Of note, V. Polishchuk (NULES) informed the team that, currently, data on wild boar occurrence and the correct border coordinates of hunting businesses are not reflected in official statistical reports, which are submitted annually to the State Statistics Service of Ukraine. In addition, the COVID-19 pandemic has further hampered efforts to understand the real-time ASF situation in the country. Thus, emphasis should be placed on enhancing data acquisition once COVID-related restrictions have lifted to ensure immediate assessment of Ukraine’s ASF crisis and, thereby, rapidly inform decision-making on measures to mitigate the disease.

Lastly, it’s also worth noting that the UP-10 team’s choice of locations and timeframes for market sampling benefited from historical analyses of ASF

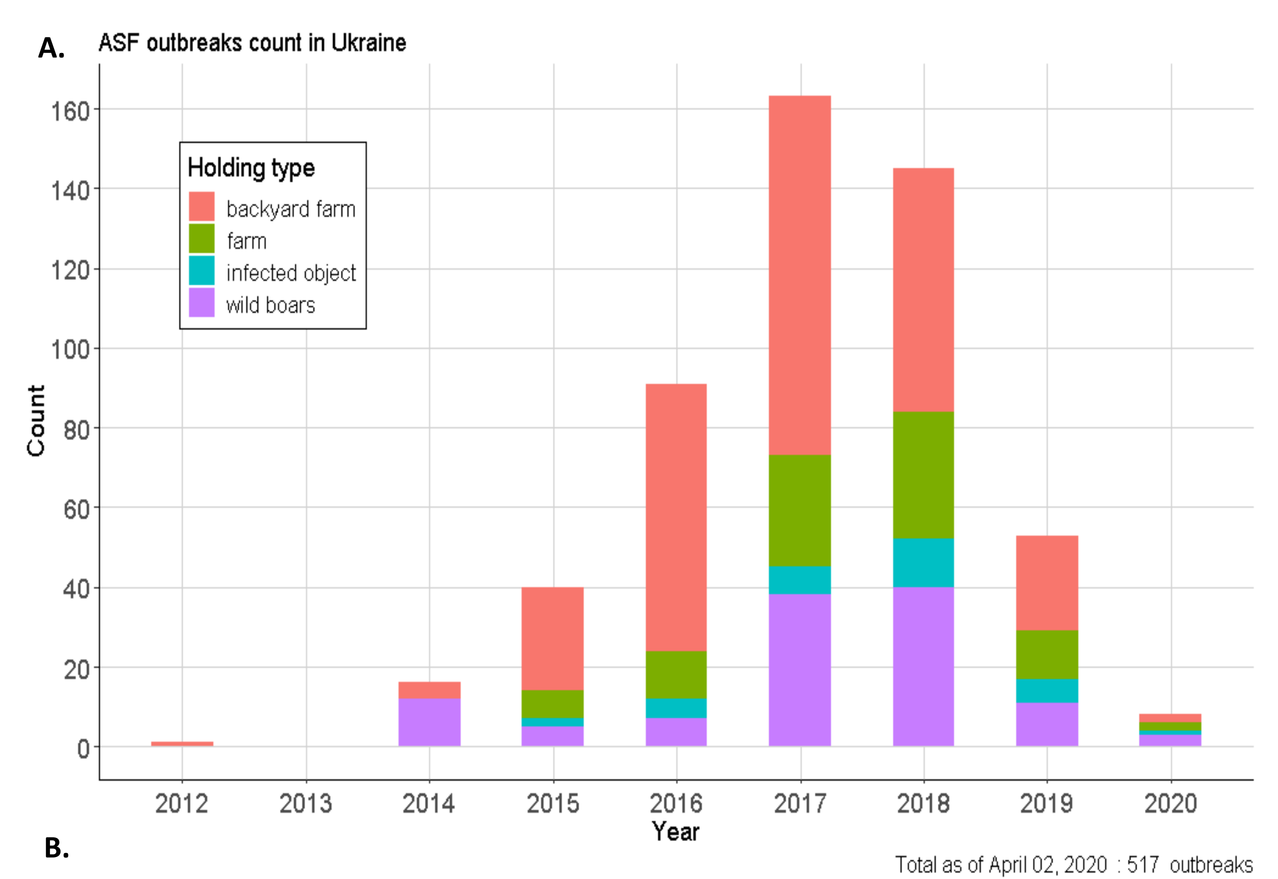
outbreaks and occurrences that have been tracked by the SSUFSCP since 2012, which provided valuable insight into seasonality and afforded access to regional cues relevant to site selection. The ability to locate positive samples demonstrated both the importance of the dataset collected by the SSUFSCP and the need to continue to build upon these efforts in future iterations of this programmatic work.



Species	Number of ASF Outbreaks per Year								
	2012	2013	2014	2015	2016	2017	2018	2019	2020
Domestic Pigs	1	0	4	35	84	125	105	42	5
Wild boar	0	0	12	5	7	38	40	11	3

Figure 3. Example of using ggplot2 package in R to show (A) number of annual ASF outbreaks in domestic pigs (red) and wild boar (blue), 2012-02 April 2020, correlated (B) data for each year (also presented in the UP-9 OY1 Final Report).

A.



B.

Species	Number of ASF Outbreaks per Year								
	2012	2013	2014	2015	2016	2017	2018	2019	2020
Domestic Pigs-Backyard Farm	1	0	4	26	67	90	61	24	2
Domestic Pigs-Farm	0	0	0	7	12	28	32	12	2
Domestic Pigs-infected Object	0	0	0	2	5	7	12	6	1
Wild Boar	0	0	12	5	7	38	40	11	3

Figure 4. Example of using ggplot2 package in R to show (A) number of annual ASF outbreaks in domestic pigs according to holding type and in wild boar (2012- 02 April 2020), correlated to (B) data for each year.

Figure 5. Example of using ggplot2 package in R to show epidemiological curve in domestic pigs (red line) and wild boar (blue line) by month since 2014. Dashed lines describing the temporal trend were calculated with LOESS smoothing. The temporal distribution of the outbreaks in wild boar peaked later than in domestic pigs (also presented in the UP-9 OY1 Final Report).

A.



B.

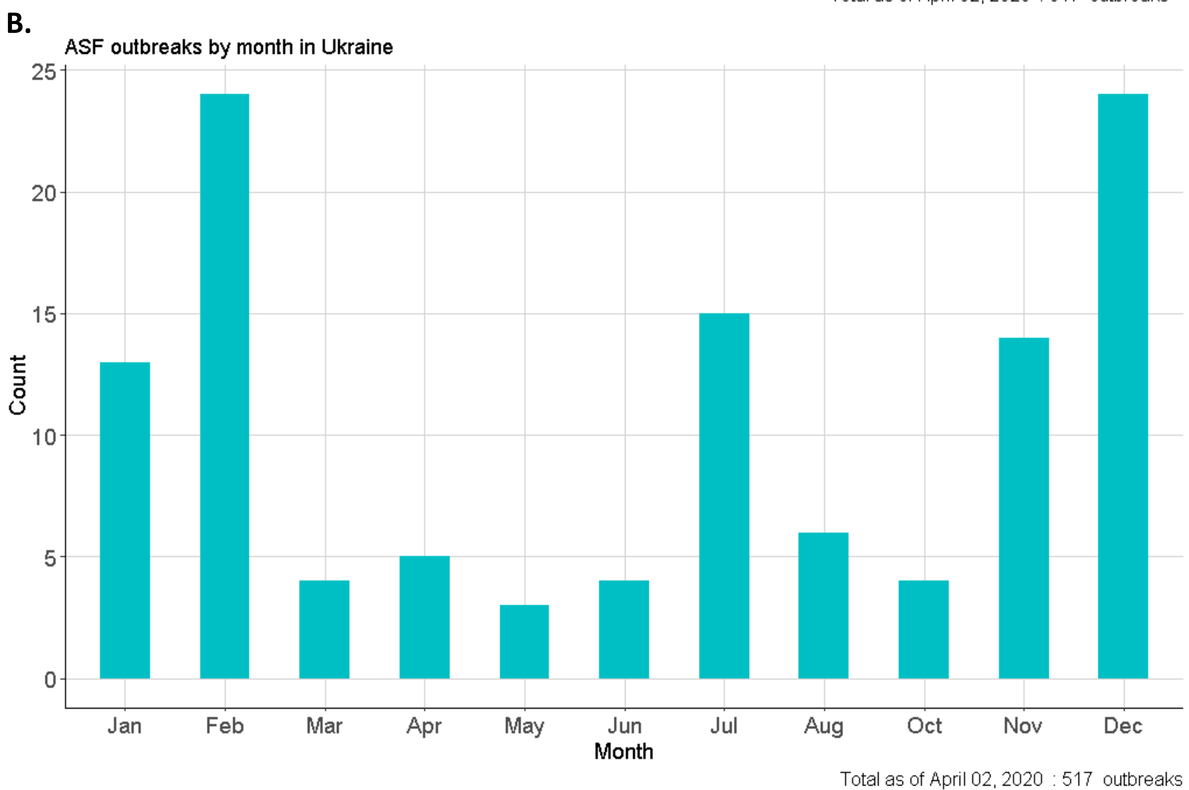


Figure 6. Example of using the ggplot2 package in R to show the monthly distribution of ASF outbreaks, from 2012 to 02 April 2020, in (A) domestic pigs and (B) wild boar. The highest number of outbreaks were detected in domestic pigs in July, August, and October; whereas, the highest number in wild boar were detected during winter months and in July.

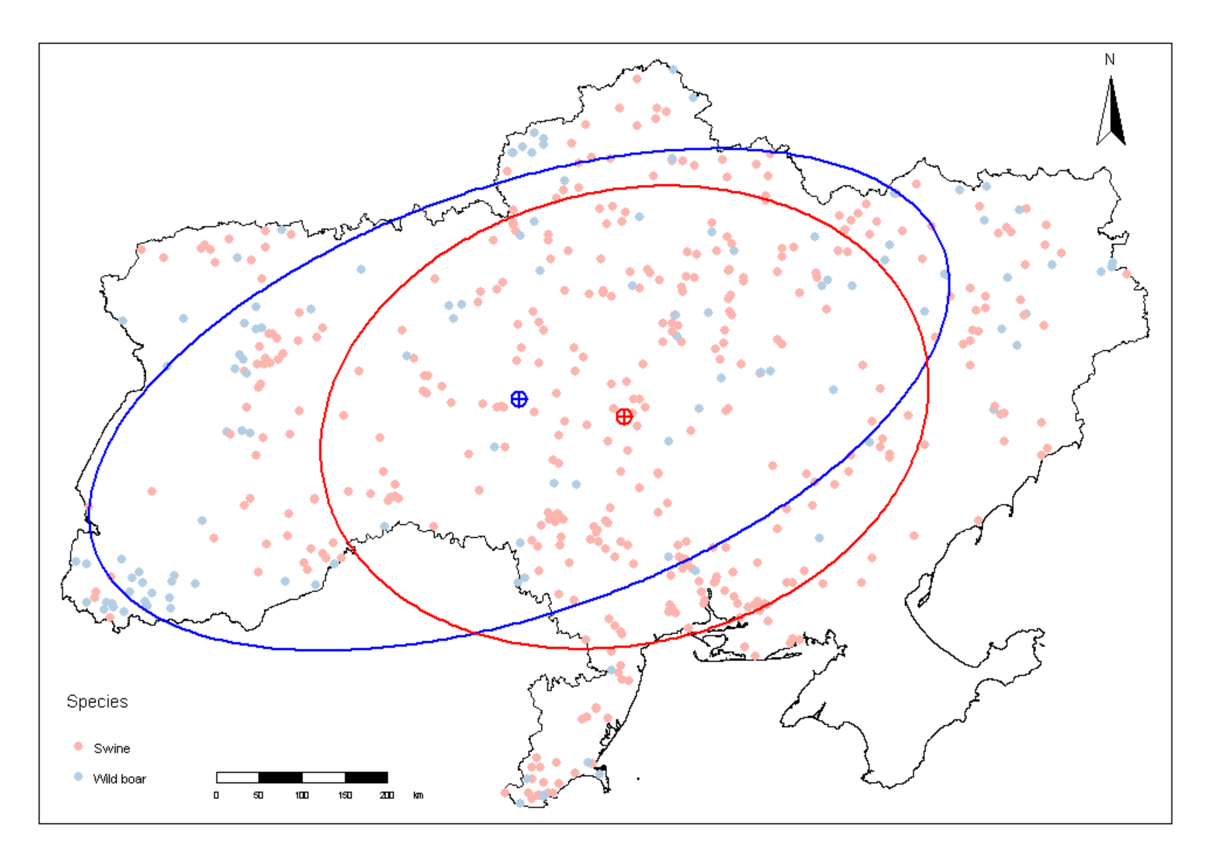


Figure 7. Example of using the aspace and tmap packages in R to show the distribution of ASF outbreaks in Ukraine, 2012-02 April 2020. Red and blue dots represent locations where outbreaks occurred in domestic pigs and wild boar, respectively; blue and red cross-hatched circles represent the mean center of each distribution; and blue and red ellipses represent the standard deviational ellipse of each distribution.

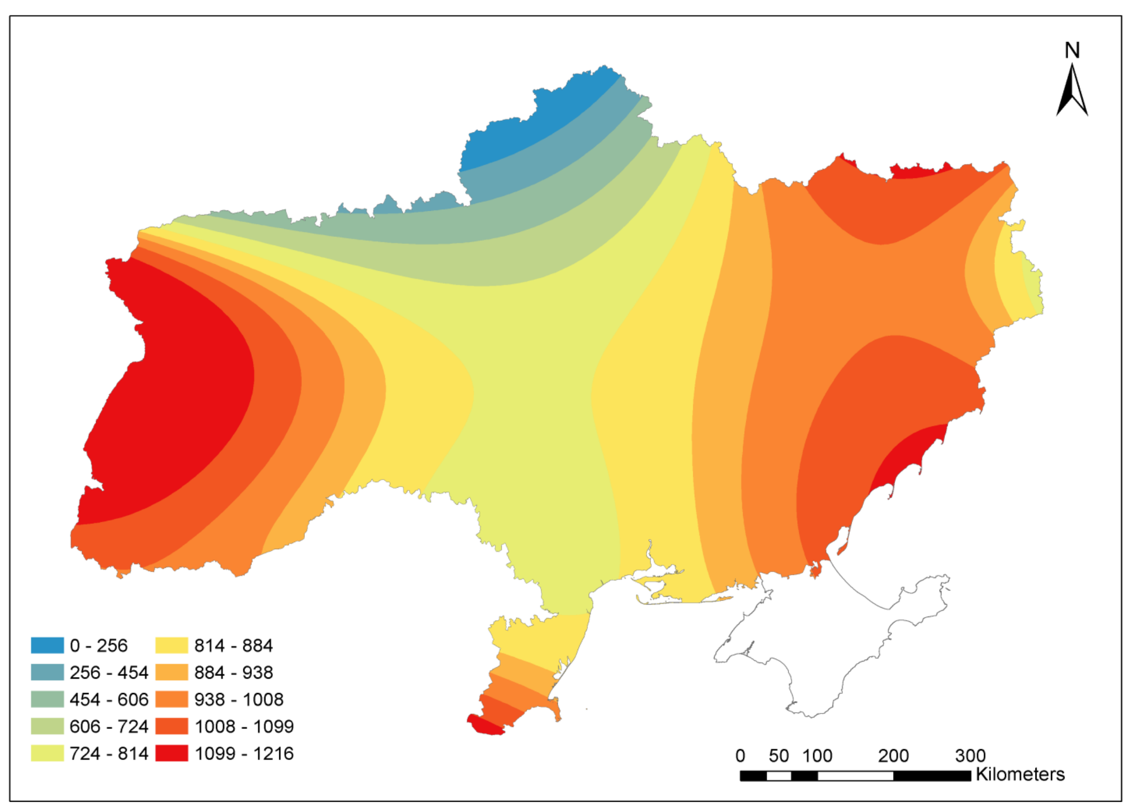


Figure 8. Example of trend surface analysis, with colored surface showing average diffusion of ASF outbreaks in space and time (days from the first outbreak); earliest and latest outbreaks are blue and red, respectively.

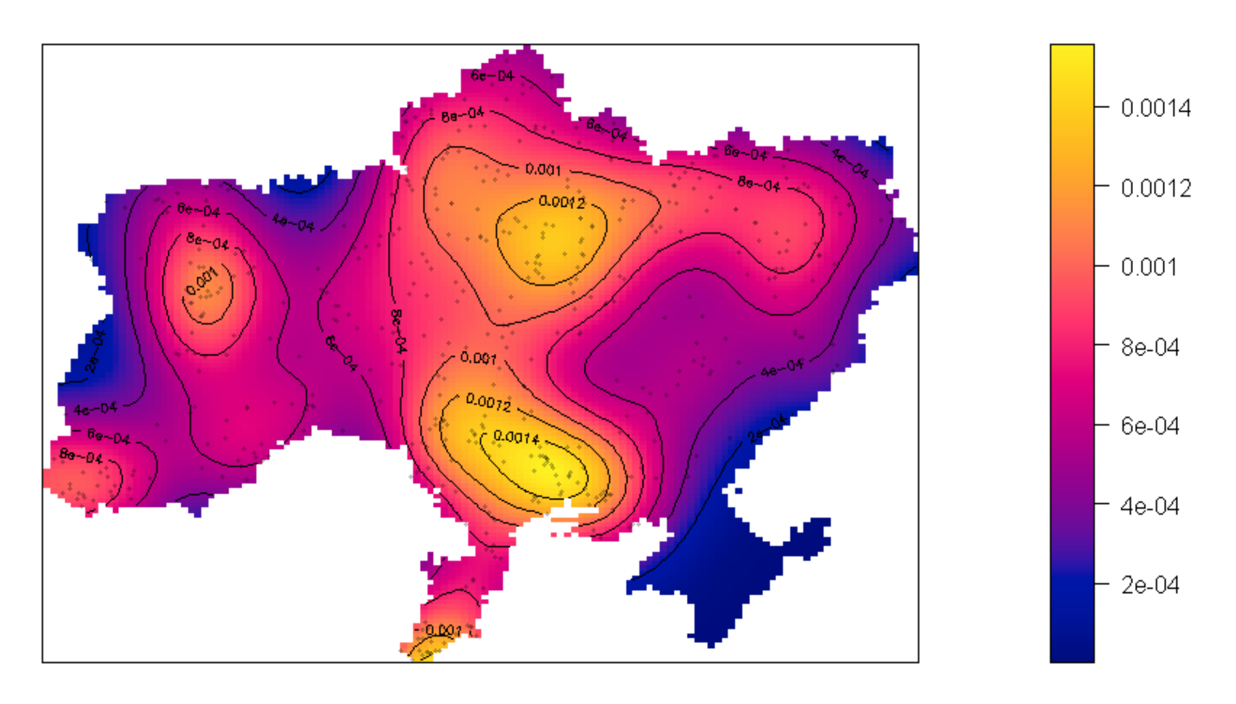


Figure 9. Example of using KDE to show the density of ASF outbreaks per square kilometer in both wild boar and domestic pigs since 2014 (also presented in the UP-9 OY1 Final Report).

GOAL 2. Track anthropogenic and socio-economic factors

Results and Discussion on Tasks 2.1: Ensure proper protocol and biosecurity throughout sample collection, shipping, and testing.

Results:

- A week-long intensive **BS&S Workshop** was conducted (Kyiv, Ukraine; 17-21 February 2020). The first three days of the Workshop engaged Oblast-level SSUFSCP representatives in identifying and evaluating the risks that they encountered in pop-up pork markets during data collection. As described below, the UP-10 training team provided participants with supplementary training on livestock disease threats and emergency response measures; while, participants provided feedback on current policies and protocols in place across Ukraine for animal disease response, including gaps and challenges. Activities were also conducted regarding measures that must be taken after an outbreak, including cleanup and recovery of infected animals. A summary of the team's efforts are as follows:

    - S. Higgs and D. Vanlandingham (KSU) provided trainings on control of livestock diseases, with special emphasis on ASF. C. Beardsley (KSU) administered animal disease response training, an OIE-based awareness course for non-traditional responders, and he also provided orientation on OIE regulations regarding exports, then led discussion on potential impacts to international trade. Using ASF as the disease of interest, the group discussed risk-based analysis needed in response, with examples of how the Ukraine government handled payouts for contaminated pig culling (reasonable market price, partial compensation, etc.), animal disposal, and quarantine during restocking. V. Polishchuk (NULES) provided informative insight.

    - Presentations included a discussion on the role of wild boar/feral swine in ASFV spread and maintenance, hunter responsibilities, and the impact of ASF on domestic swine production and associated economic impacts, with examples from other countries (e.g., ASF China and classical swine fever in the Netherlands). D. Vanlandingham presented information on the detection and surveillance of foreign animal diseases, with examples provided for additional pathogens of concern (West Nile virus and chikungunya virus). C. Beardsley's presentation focused on the control of ASFV: Risks and considerations related to animal movement, quarantine, road closures, public awareness campaigns, and protective behavioral change communications.

    - These presentations led naturally into group discussions concerning how ASFV cases are reported in Ukraine. Karen Saylors (Labyrinth Global

Health) facilitated a discussion with participants regarding communication, inviting them to speak about the rules of animal case reporting and how this influences producers (small holders and commercial farms), animal transporters, and the economies of local communities. Participants extensively discussed quarantine rules and potential interventions around international travel, commerce and economic development, and public health measures. C. Beardsley provided information pertaining to measures that must be taken after an outbreak, specifically around cleanup and recovery of infected property, which sparked discussion concerning how pig carcasses are handled. In Ukraine, the most common accepted approach is by burning carcasses, and the group discussed how this affects animal farmers, their reactions about compensation, and how Food Safety authorities manage cases in their different Oblasts.

  - The last two days of the workshop included representatives of livestock workers, a pig breeder association, and SSUFSCP leadership, as well as two FAO representatives. The project trainers presented policies for control and containment of ASF, lessons learned from other animal disease outbreaks, reflections on economic impacts associated with outbreaks, as well as the importance of public awareness and communication.

- In support of initiating sample collection activities, a thorough review of the efficacy and clarity of existing protocols was conducted during project team visits to each target Oblast. Regional sample collection teams were trained on standard operating procedures (SOPs) for sample collection, packing, and shipping to ensure that protocols and biosecurity measures were understood and followed.

- V. Polishchuk provided app training to all individuals selected to buy meat for analysis and data collection.

- During initial visits to each target Oblast, study teams adjusted the data entry platform, schedule, and approach for purchasing pork samples at selling points.

Discussion:
The group reviewed UP-10 online data and conducted group analysis, with project teams from Kharkiv, Odesa, Zakarpattia, and Rivne Oblasts sharing their observations concerning perceived risks at illegal pork selling points, as well as discussing lessons learned and making policy recommendations about illegal pork markets and sales points. Facilitators invited Oblast teams to discuss the rules of animal case reporting and how these influence producers, animal

transporters, and the economies of local communities. Details emerged regarding State quarantine rules, including elements that are problematic for farmers, as well as potential interventions around international trade, animal movement, and public health measures. There was extensive discussion on the handling of infected pig carcasses, as well as the accepted approaches in Ukraine, realities and reactions about compensation for losses, and how Food Safety authorities manage cases in their different Oblasts.

Workshop participants considered the question: “Is eradication possible?”. FAO representatives pointed out the vulnerability of backyard farms as these are where most outbreaks happen, and they emphasized the importance of local governments’ involvement, pointing out that very little educational information is provided to farmers and traders in rural areas, which exacerbates risk. FAO highlighted this point by explaining that there are 1,200,000 backyard farms in Ukraine, which is where outbreaks are most prominent and, thus, should be the focus of control strategies. In this regard, there is a need for better/quicker compensation for small farms. Several participants explained that the compensation process is too complicated, with too many prerequisites needed to be met for obtaining full compensation. This challenge must be overcome to ensure farmers are motivated to report sick or dead animals. Compensation of pig owners must involve agro-industry. One participant mentioned that Ukrainian guidelines do not comply with European directives, so there is a need for policy advocacy.

Lastly, Workshop participants were organized into regional working groups and discussed priorities in their region, giving consideration to outstanding gaps and priorities that could be potentially addressed via a follow-on project. Highlighted suggestions included further engagement with stakeholder associations (hunters, small holder pig farmers, etc.), further surveillance of anthropogenic behavioral risk factors, and more intensive risk communication to hard-to-reach populations. Another topic raised was further investigation of co-infection with other DTRA priority pathogen diseases such as anthrax, brucellosis, or classical swine fever.

Results and Discussion on Tasks 2.2: Analysis of biological samples of pork products collected at non-licensed points of sale by project stakeholders to test for the presence of ASFV.

Results:

- Tasks 2.2 a-b: The project’s biological sample collection strategy was developed and implemented. Sample collection was finalized in each target Oblast (**Figure 10**). All samples were delivered to SSRILDVSE for subsequent ASFV analysis using PCR methods (**Table 3**).



Figure 10. Highlighted territories for sample collection activities.

Table 3. Targeted Oblasts and number of samples collected, then delivered to SSRILDVSE for analysis.

Oblast	Number of samples
Zakarpattia Oblast	1000
Odesa Oblast	1000
Rivne Oblast	1000
Kharkiv Oblast	1000
Total	**4000**

Tasks 2.2 c-e: Analysis of samples by PCR for ASFV nucleic acid detection was initiated in Q4. SSRILDVSE tested 4,000 samples from four Oblasts of Ukraine for the presence of ASFV DNA by real time PCR using the Belarusian-produced test system from Sivital (ТУ BY 391360704.011-2015). This test system consists of two kits for (a) DNA extraction and (b) detection of viral DNA by real-time PCR (See Appendix M for the kit protocol). A single standardized approach was used for DNA extraction, with all samples processed according to the kit protocol for Isolation of DNA from tissue samples, meat products, and whole blood (columnar method). ASFV DNA was detected in 8 samples (4 from Odesa Oblast, 2 from Zakarpattia Oblast, and 2 from Kharkiv Oblast). For confirmation, the starting material for each positive sample was retested using existing SSRILDVSE protocols for nucleic acid extraction and real-time PCR via the LSI VetMAX™ African Swine Fever Virus Detection Kit. Detailed information regarding the samples and laboratory findings are presented in **Table 4** below.

Table 4. Laboratory results for PCR detection of ASFV-positive samples[1].

№	Sample designation/type of sample	Test results using SIVITAL ASFV Real-Time PCR Kit (Ct value[2])	Test results using LSI VetMAX African Swine Fever Virus Detection Kit
Odesa Oblast			
2	1356188/muscular tissue	32.31	28.10
	1356192/fat	31.43	26.4
Positive Control		28.40	25.46
Negative Control		-	-
6	57448/ muscular tissue	35.10[3]	27.14
Positive Control		28.60	25.93
Negative Control		-	-
6	57024/ muscular tissue	34.30	28.64
Positive Control		29.03	25.93
Negative Control		-	-
TOTAL		**4**	**4**
Zakarpattia Oblast			
5	1351477/muscular tissue	29.01	24.94
	1351767/fat	28.20	23.71
Positive Control		28.14	24.42
Negative Control		-	-
TOTAL		**2**	**2**
Kharkiv Oblast			
1	1353803/muscular tissue	33.37	27.45
2	1353982/muscular tissue	31.72	23.70
Positive Control		32.70	23.92
Negative Control		-	-
TOTAL		**2**	**2**

[1] All data for the above table were conveyed to SMEs by Ukrainian project participant M. Sapachova, SSRILDVSE.
[2]Ct - threshold cycle (cycle number) obtained during amplification of the tested DNA samples.
Each cycle of DNA amplification leads to the generation of a fluorescent reporter dye signal measured in the FAM (Green) channel for the target and the HEX (Yellow) for the internal control. If Ct ≤ 40 in FAM channel, and the Ct < 40 in HEX channel, then tested DNA contains ASFV genome fragments (positive). Positive and negative controls were provided by the kits.
[3] Sivital data from initial testing of sample; at the time of this submission, data for retested sample were not provided by SSRILDVSE, but sample was confirmed positive by LSI.

Discussion:

SSRILDVSE is the only laboratory with official accreditation in Ukraine for ASFV analysis using PCR methods (**Table 3**). To ensure that project results can be officially used by the competent authority, the project's collaborators agreed to deliver all samples to SSRILDVSE's Virology Lab where analysis was to be conducted using the Sivital test-kits, with confirmatory testing of positive samples carried out via the officially registered LSI kit. Prior to study launch, two experts from Sivital administered in-person training at SSRILDVSE's Virology Lab, thereby demonstrating PCR testing and analysis for all UP-10 participating institutes (Kyiv, Ukraine; 24 January 2020). While it was agreed that all tests would be performed at ILD, the test-kits were distributed among SSRILDVSE and the project's two NAAS Institutes (IVM and IECVM) to permit specialists from each of the participating institutes opportunity to perform testing. In this regard, each institute received a portion of the samples collected and stored at SSRILDVSE. While IVM was able to finalize analysis of their provided samples, IECVM was unable to complete testing due to challenges stemming from the COVID-19 pandemic.

Although the above results are informative and confirm the presence of ASFV-infected pork products available for purchase at illegal sales sights, a significant finding, unexpected knowledge gaps in laboratory analysis were identified upon the SME's receipt of the dataset from SSRILDVSE, which reduced the impact of the study's findings. As described below, several missteps rendered the PCR data less than complete, serving as retrospective lesson to the laboratory staff involved in the study.

The following issues were identified upon review of the dataset and via interviews with laboratory staff:

- Sample type was not recorded by the lab or considered when preparing nucleic acid extractions.
- Although sample types included raw meat, raw organ meat, raw salo, smoked salo, smoked meat, and sausages, a single protocol was utilized for all samples, and no controls were performed to validate that a single extraction method was suitable for all sample types.
- No quality control was applied to the collection of data or for normalizing nucleic acid concentrations across the different sample types.
- No known positive samples collected as part of the regular state surveillance program were tested to validate the sensitivity of the applied method or ability of the protocol to provide positive samples from non-routine tissue types.

Collectively, these protocol issues undermined the usefulness of this analysis and prevented opportunity for a thorough comparative assessment between the LSI

test kit and the Sivital detection platform, thereby obviating the potential for replacing the former kit with the latter, less expensive and regionally-produced, platform. Based on review of confirmed data though, the Sivital test kit yielded results that aligned with the LSI test kit for ASFV-positive samples. Furthermore, inquiries addressed by the project's participants and review of laboratory records allowed for the identification of the most probable sample type for each of the confirmed positive samples. Despite such observations, these data were retrospectively added, and thus, the certainty of study findings is insufficient for validation of the dataset. Considering that 2 of the study's 8 positive samples were derived from fat, and no organ meat or prepared products were positive, the standardized approach used for analysis of all samples, which did not account for adjustments to protocol parameters based on sample type, call into question study results. Unfortunately, by applying the high-throughput testing principles typically implemented by the state surveillance system, the laboratory failed to demonstrate a deep understanding of experimental design or ability to shift perspective to accommodate for a rigorous scientific study.

Based on the qualitative nature of this analysis, the study does confirm that ASFV positive samples exist within Ukraine's illegal trade network and highlights the potential for anthropogenic dissemination of ASFV-infected materials via illegal markets. However, no quantitative conclusions should be drawn from these findings related to either the rate of occurrence of such samples in the illegal trade network or the confirmed contribution of these products to the furthering of infection in domestic or wild animals.

Taking into consideration the study's findings, it behooves the SSUFSCP and SSRILDVSE to complete evaluation and validation of the Sivital ASFV kit (ТУ BY 391360704.011-2015) for official use within the Ukrainian veterinary biosurveillance system, as the platform is cost effective and can be easily procured due to the regional proximity of the manufacturer in Vitebsk, Belarus.

Results and Discussion on Tasks 2.3 and 2.4:

- Task 2.3. Demonstrate and document anthropogenic factors contributing to the spread of ASF in Ukraine and the need to implement effective biosecurity and control measures for preventing farm-to-farm and farm-to-wildlife spread.

- Task 2.4. Assess the relative risk of ASFV spread within Ukraine and across regional borders via commercial trade routes of pigs and pig products, the illegal distribution and transport of pigs and pig products, and wild boar movements.

Results:

- Task 2.3 a-b: To analyze anthropogenic factors associated with the spread of ASF, project stakeholders collected meat sampling data, which are stored in the database created by V. Polishchuk (NULES). Analyses of data collected are shown below (**Tables 5-9**). By questioning sellers at illegal markets, project team members determined that backyard farms serve as the primary source of pork products at such markets. The origin of pork products sold at illegal/unofficial outlets in each Oblast is shown below in **Table 5**.

Table 5. Origin of pork products purchased at the illegal/unofficial outlets surveyed in each target Oblast.

Pork products sold in:	Pork products delivered from:	Number of samples
Zakarpattia Oblast	Zakarpattia Oblast	1000
Odesa Oblast	Odesa Oblast	1000
Rivne Oblast	Volyn Oblast	1
	Zhytomyr Oblast	2
	Rivne Oblast	985
	Ternopil Oblast	10
	Khmelnytskyi Oblast	2
Kharkiv Oblast	Poltava Oblast	2
	Sumy Oblast	4
	Kharkiv	994
Total Samples Purchased		**4000**

As noted above, the majority of meat samples purchased in the four Oblasts originated from backyards. For Rivne and Kharkiv Oblasts, vendors reported meat originating from pigs raised in multiple neighboring Oblasts (**Table 5**). Based on this survey, backyard farmers, as well as vendors serving as a go-between for farmers and purchasers, are involved in illegal meat trading, which thereby broadens the conduit for ASFV circulation and spread across Ukraine.

Table 6. Number of sellers distributed by age in each Oblast.

Age	Number of Sellers			
	Zakarpattia Oblast	Odesa Oblast	Rivne Oblast	Kharkiv Oblast
> 60 years old	256	27	254	62
40-60 years old	583	627	511	849
20-40 years old	161	338	234	89
0-20 years old	0	8	1	0
Total	**1000**	**1000**	**1000**	**1000**

Table 7. Distribution of sellers wearing gloves or not wearing gloves nor other forms of personal protective equipment (PPE) in each Oblast.

PPE	Number of Sellers			
	Zakarpattia Oblast	Odesa Oblast	Rivne Oblast	Kharkiv Oblast
Sellers without PPE	1000	824	960	964
Sellers with gloves	0	176	40	6
Total	**1000**	**1000**	**1000**	**1000**

Table 8. Meat and pork product storage locations where samples were purchased.

Locations of meat and pork products at the selling point	Number of Sellers			
	Zakarpattia Oblast	Odesa Oblast	Rivne Oblast	Kharkiv Oblast
On the table or counter	724	694	435	968
In a bag or via a mat on the ground	275	266	565	6
In a car trunk or trailer	1	40	0	26
Total	**1000**	**1000**	**1000**	**1000**

Table 9. Summary of characteristics related to sites where positive samples were sold.

Oblast	Seller's gender	Estimated seller's age	Origin of pork	Place of sale	PPE	Other products than pork
Zakarpattia Oblast	Woman	40 - 60 years	Homegrown	Bag or mat on ground	No	No
	Woman	40 - 60 years	Homegrown	Bag or mat on ground	No	No
Odesa Oblast	Woman	40 - 60 years	Homegrown	Counter or table	No	Salo (fat)
	Woman	20 - 40 years	Homegrown	Counter or table	No	Salo (fat)
	Man	40 - 60 years	Homegrown	Bag or mat on ground	Yes	Salo (fat)
	Woman	40 - 60 years	Homegrown	Counter or table	No	Tenderloin, head
Kharkiv Oblast	Woman	40 - 60 years	Homegrown	Counter or table	No	Sausage
	Woman	40 - 60 years	Homegrown	Counter or table	No	Salo, head, limbs (legs)

Discussion:
Analysis of the number of non-licensed pork products selling points in four Oblasts, the nature and volume of the products sold at these sites, and data obtained during interviews with pork sellers provided the basis upon which to inform understanding of illegal trade routes in Ukraine and of the sellers' general profile, which will help guide educational and outreach activities. These insights, coupled with laboratory data of collected samples, illuminate the potential for disease transmission through the country's illegal trade routes ("from the field to the table"), which, in turn, compel consideration of effective strategies for the control of ASF, including strengthening authorities' approach to quarantine measures. Due to the aforementioned limitations in sample testing though, correlations between positive samples and selling points cannot be made at this time, further prompting continued investigation.

Of note, the observations made by the field groups are novel for a study of this nature in Ukraine. Through the inclusion of local-level epidemiologists and community members in the study groups, UP-10 contributed to building understanding of the nature of illegal sales and their potential contribution to the spread of human and animal disease by which local perceptions can be shifted. The ease of finding illegal sales points, coupled with the comfort level of local communities supporting these sellers, raises concern. In this regard, the project's collective findings reinforce the need to expand enforcement of regulations, the range of the biosurveillance network, and activities in support of public education and outreach.

GOAL 3. Public policy and communication through training, education, and outreach

Results and Discussion on Tasks 3.1: Establish a GIS and Computational Short-Term Modeling Fellowship.

Results:
A call for GIS and Modeling Fellowship applications was announced on 26 November 2019 and ran through 06 December 2019. Based on the applications received, Maksym Bezymennyi, a researcher in the Department of International Activity and GIS at IVM, was selected as the Fellow for this opportunity. The GIS and Modeling Fellowship was arranged in collaboration with the University of Florida, Gainesville. Due to the COVID-19 pandemic, M. Bezymennyi had to reschedule his return to Ukraine from 20 April to 12 May 2020, which offered him additional opportunity to work with University experts, notably Jason Blackburn, University Director of the Spatial Epidemiology and Ecology Research Laboratory (Gainesville, FL).

Please refer to Section 2.6.1, Goal 1-Tasks 1.1 and 1.2, for in-depth description of his Fellowship activities and outputs. In addition to noted efforts, M. Bezymennyi prepared several presentations for training sessions on spatial analysis, which he administered to local participants upon his return to Ukraine with the support of Train-the-Trainer (T3) candidate Iryna Makovska, a PhD student from the Department of Epizootology, Microbiology, and Virology at NULES.

Discussion:
By leveraging the project's Fellowship, UP-10 was able to effectively develop and graduate two mature T3 candidates, who demonstrated their ability to train UP-10 participants as well as individuals enrolled in DTRA's Biological Threat Reduction Integrating Contract (BTRIC) training program. M. Bezymennyi applied his tenure at the University of Florida to interact directly with US-based faculty and members of their research groups, which equipped him with a deeper understanding of new approaches to education and also US-based research programs. Advancement of his technical knowledge base supported his ability to ultimately serve as a lead trainer. Upon his return to Ukraine, M. Bezymennyi was able to directly transfer this new understanding to junior T3 candidate I. Makovska. In a first for the BTRP-Ukraine training program, the two local instructors went on to deliver training as a team without the assistance of other trainers. The UP-10 project team believes that the Fellowship demonstrated a successful approach for advanced training in research methodology, management of research groups, and administration of training; ultimately proving the Fellowship's merits as an educational opportunity unattainable solely through in-country based training events.

Results and Discussion on Tasks 3.2: Develop training curricula for GIS and perform outreach to inform local, regional, and national policy development.

Results:
Two GIS training events were organized by project team members.

The first training event (in support of Task 3.2.b) was entitled "Introduction to GIS environment and spatial-temporal analysis", which was implemented by SAFOSO SMEs (Kyiv, Ukraine; 25-27 February 2020) with assistance provided by T3 candidate I. Makovska, who moderated the practical session.

The main training objectives were:

- Introduction to GIS environment.
- Introduction to Spatial Analysis (basic-intermediate level).
- Learning to use of key functionalities on selected software (QGIS, SatScan, etc.).
- Discussion on the use of GIS and Spatial Analysis.

Trainees were professionals from the project's collaborating institutes: IVM, SSCIBSM, IECVM, SSRILDVSE, and NULES. Participants attended lectures and practical sessions pertaining to various methods for spatial and temporal analysis, from simple visualization of disease cases to more advanced methods, such as extraction mapping, spatial correlation, and clusters identification. All hands-on practical sessions were implemented using free software. The agenda and other training-related materials are available via the UP-10 website at: http://www.up10.vet.ua/index.php/purpose-project/324-introduction-to-gis-and-the-basics-of-spatial-analysis

The second training event (in support of Task 3.2.c) was entitled "The GIS Environment: How to make the best use of maps", which was implemented virtually due to the COVID 19 pandemic (01 July 2020).

The main training objectives were:

- Introduction to GIS framework and spatial analysis.
- Understanding the role of spatial analysis for planning control and surveillance programs.

Participants were policy makers and risk managers from SSCIBSM, SSRILDVSE, IVM, NULES, and IECVM. Trainees accessed audio-video materials (prepared by SAFOSO) on GIS and Spatial Analysis, reviewed published papers, attended lectures, and actively participated in virtual discussions on how to interpret spatial analysis outputs in the context of infectious disease control strategy. At the conclusion of training, participants were able to critically review and comment on published spatial analysis papers. The list of trainees is presented in Appendix K.

Discussion:
Both training events were well received, equipping trainees with the skills necessary to further develop and utilize spatial analysis and GIS in epidemiologic research and risk management. Participants at both training events acquired knowledge for performing spatial analysis methods and recommending appropriate risk management measures based on outputs. This achievement is particularly important as GIS and spatial analysis are powerful tools for enhancing the ability to investigate epidemiological patterns of infectious diseases as a means to devise more effective disease control plans.

Results and Discussion on Tasks 3.3: Develop audience-appropriate materials to support education and public outreach strategies.

Results:

- Materials for the two GIS training events (see Task 3.2) were produced and shared with project team members and other training attendees.

- Educational materials and target groups (**Table 10**) were identified by training participants.

Table 10. Key target groups and type of outreach materials.

Target group	Geographical coverage	Number of beneficiaries reached	Stakeholders involvement	Educational material
Local authorities	All Ukraine	All rayons	Rayon state administration departments	Social gatherings - explanatory work on collecting and analyzing data (where to look for information?); administration site
Backyard farms	Oblasts selected within the project: Zakarpattia, Kyiv, Odesa, Rivne, Kharkiv	All Rayons	Association of swine breeders of Ukraine	Information calendars (wall, small); newspaper distribution in mailboxes
			Oblast authority of SSUFSCP	Wall posters in the village council; Small things in the private household (disinfectants, disposable paper towels, wet wipes, plastic buckets, protective clothing, etc.), with a logo or stickers conveying website address
			Village council	
Hunters	All Ukraine	All hunting grounds (owners-lessees)	SFRA	Warning signs in places of recreation (recreational areas for picnics, refueling stations, dressing rooms) and at the entrance to hunting grounds

Target group	Geographical coverage	Number of beneficiaries reached	Stakeholders involvement	Educational material
			Hunting and Fishing Association of Ukraine	Information cards issued together with licenses for hunting; trainings for owners of hunting grounds; trainings for hunters; messages in professional journals
Local-level slaughtering facilities	Oblasts selected within the project: Zakarpattia, Kyiv, Odesa, Rivne, Kharkiv	All Rayons	Oblast authority of SSUFSCP, village council	Information posters for distribution; protective clothing with stickers; plastic buckets and disinfectants, with the logo or stickers conveying website address
Commercial farms	All Ukraine	All registered farms	SSUFSCP	Trainings for veterinarians/ managers
			Pig associations	Social networks and groups (FB, Viber, Messenger, Telegram, etc.)
Public	Oblasts selected within the project: Zakarpattia, Kyiv, Odesa, Rivne, Kharkiv		Local authorities (city hall, Oblast council)	Handout materials (calendars, brochures, etc.)
			Protection on Consumer Rights	eco-bags and/or canvas backpacks
			SSUFSCP	Social advertising in the subway (up to 1 min. free of charge)
			Media sources	

Target group	Geographical coverage	Number of beneficiaries reached	Stakeholders involvement	Educational material
Traders (meat & animals)	All Ukraine	4 oblasts	SSUFSCP	Information signs (billboards, light boxes, banners) on roads/highways
			Vendors in places where livestock products are sold	Posters in places where meat products are sold
				Educational work with the population (backyards)
Students	Oblasts selected within the project: Zakarpattia, Kyiv, Odesa, Rivne, Kharkiv		Student associations	Mouse pads
			Dean's office	Pens; calendars; laptop stickers; badges; social networks; online platform for learning and communication
Trainers (for official vets)	All Ukraine	All oblasts; main epizootologists; lab specialists; research institute representatives; professors at vet and agricultural faculties	SSUFSCP	Preparation of presentation templates; participation in international events (conferences, seminars, simulation exercises); trainings; E-learning

- It was proposed that as a part of future work that educational materials for the general public be developed based on the analysis of the pork collected from the markets.
- Methodology learned through implementation of the project's KAP survey (see Task 3.4.2) has been incorporated into the NULES

institutional training program entitled "Information Technology in Veterinary Medicine" for achievement of master-level degrees in the specialties "Veterinary Medicine" and "Veterinary Hygiene, Sanitation and Expertise". The UP-10-based 2-hour course provides a practical lesson in the methodology of the KAP survey and also offers research protocols for using computer programs for statistical research. This will allow future veterinarians to gain deeper understanding of the tools needed for epidemiological surveys, including methods for investigation of outbreaks, collection of epidemiological data and analysis of indicators using specialized software, the basics of evaluation and informed management decisions, and critical evaluation of published information.

The Link to the program on the official NULES website is provided below: https://nubip.edu.ua/sites/default/files/u228/robocha_programa_informaciyni_tehnologiyi_u_vet._medicini_magistri_211_i_212_2020_r.pdf

Discussion:
Throughout UP-10, previously developed materials were reviewed, and gaps where the addition of new materials would be beneficial were discussed. The unique multi-stakeholder discussion group format of UP-10 project meetings ensured that multiple voices and perspectives were effectively expressed and captured in planning for future projects and outreach campaigns, as well as in recommendations provided to the Government of Ukraine (GoUA).

While much progress was made in support of this Task, efforts were significantly impacted by work stoppage experienced in the project's final months due to the COVID-19 pandemic. For many of the outreach and policy objectives, seminal meetings and planning sessions were scheduled to take place at the 2020 BTRP Regional One Health Research Symposium. However, following cancellation of the Symposium and enforcement of quarantine measures, team members were unable to conduct the large group meetings required to finalize this and other project Tasks. That being said, significant groundwork was laid by the UP-10 project team thereby either informing future GoUA initiatives or serving as a launch pad for future CBR activities that include expanding public outreach and education.

Results and Discussion on Tasks 3.4: Educate and perform outreach to inform local, regional, and national policy development.

(1) Results and Discussion on Task 3.4.1: Outreach efforts

Results:
A Public Outreach Working Group was established (Task 3.4.1 a) via a SAFOSO-led in-person workshop (Kyiv, Ukraine; 12-13 June 2019), which supported Task

3.4.1.b-d. During this event, the newly formed Working Group discussed previous outreach activities on ASF implemented in Ukraine, reviewed the current epidemiological situation in Ukraine, and were introduced to the steps necessary for developing a national outreach strategy. Participants considered approaches for conducting outreach activities. The following summarizes accomplishments stemming from this event:

- Identification of potential limitations and gaps in current outreach activities to be addressed through a revised outreach strategy.

- Identification and prioritization of target groups for the UP-10 outreach strategy.
- Development of a communication plan on ASF as part of a national outreach strategy. This included consideration of arguments and messages for selected target groups.

A summary of the Public Outreach Working Group meeting is presented in Appendix J.

Discussion:
Since the beginning of the ASF crisis in Ukraine, a large number of previous outreach activities have been implemented by national and international partners. Taking this into consideration, UP-10 SMEs tried to avoid redundancy and duplication of effort. The resulting outreach implementation strategy prioritized target groups and educational materials for alternative activities implemented in the program. Local authorities, backyard farmers, and hunters were ranked as top target groups to be reached by policy and awareness campaigns. Delays in the implementation of the KAP survey (see task 3.4.2) prevented the Policy Outreach Working Group from using the KAP survey results as critical input to design the national outreach strategy with the current Ukrainian context.

(2) Results and Discussion on Task 3.4.2: KAP Survey

The aim of this activity was to identify risk factors associated with the spread of ASF in Ukraine and to understand backyard farmers' and wild boar hunters' attitudes regarding the identification and reporting of ASF suspicious cases. The KAP questionnaires were implemented in five Oblasts (**Table 11**)

Table 11. Information on the KAP Survey conducted in five Oblasts of Ukraine.

Oblast	Number of Respondents	
	Backyard Farmers	Wild Boar Hunters
Kyiv	36	50
Odesa	50	51
Rivne	47	50
Zakarpattia	50	37
Kharkiv	50	47
Total	233	235

Ukrainian researchers from IVM delivered the KAP questionnaires for Kyiv, Zakarpattia, and Rivne Oblasts while researchers from IECVM focused on Kharkiv and Odesa Oblasts. IVM and IECVM participated in piloting the Survey, in the implementation of questionnaires, and transfer of data into an electronic database.

Results:
Three KAP questionnaires were designed for wild boar hunters, backyard farmers, and small-holder farmers (Task 3.4.2 a-b; Appendix D-F). Only the questionnaires for backyard farmers and wild boar hunters were piloted (Task 3.4.2 c) and implemented (Task 3.4.2d; Appendix G-H). Data collected for these questionnaires were analyzed, and preliminary results were presented during an in-person workshop (Kyiv, Ukraine: 17-19 December 2019) (Task 3.4.2 e-g).

Results of the questionnaires distributed to wild boar hunters indicated that 76% of respondents do not feel well-informed regarding ASFV transmission, and 86% of respondents do not feel confident in recognizing the clinical signs of ASF. Furthermore, 77% of respondents have not received any training or information about wild boar diseases. Approximately, 84.8% of respondents use the wild boar they hunt for home consumption, and when they go hunting, 41% bring home leftovers whereas 28% throw the remainder into the environment. A total 46% of respondents believe that incentives serve a role in reporting. It seems that there is no stigma in relation to reporting, with 85% of respondents having said that they would encourage a hunter colleague to report a dead/sick wild boar.

Results of the questionnaires distributed to backyard farmers indicated that 53% of respondents do not feel well-informed regarding how ASFV can be transmitted, and 70% do not feel confident in recognizing the clinical signs of ASF. Furthermore, 93% of respondents have never reported a suspected case of ASF on their premises. Over two thirds of respondents (68.3%) indicated that they carry out slaughter and butchery services for the private sector. Also 79.2% of respondents mentioned doing home slaughter without veterinary inspection, and only 7% of respondents process their pigs at slaughterhouses. The most

common feeding practices of pigs entail the owner's production of pig feed (70.8%) and use of kitchen leftovers (53.6%). Trading activities of edible pork products were mentioned by 31.2% of respondents. A manuscript regarding the KAP survey for backyard farmers is in preparation.

Discussion

The UP0-10 survey results highlight the fact that wild boar hunters and backyard farmers would benefit from public outreach activities especially related to general knowledge of ASF (e.g., pertaining to the clinical signs and transmission of disease) as both target groups carry out risky activities that could facilitate the spread of ASF in the food chain and also to susceptible animals. While FSCP has indicated that such outreach activities were previously conducted and that members of the public should already be fully informed, the results of this study demonstrated the need for continued efforts and refreshing public perception about the serious nature of ASF and the activities that can contribute to the anthropogenic spread of this disease within the community and across regions. Based on input received from stakeholders and other interest groups, the majority of those participating in the UP-10 multi-stakeholder project meetings agreed that the need for additional public outreach and education identified through the project's surveys is real and highly relevant.

As with other aspects of ASF containment and control programs, additional state funding could be beneficial towards improving the situation within Ukraine. For example, despite it seeming that there is no stigma among wild boar hunters regarding reporting ASF suspected cases, incentives could play a role and increase the reporting and identification of infected wild animals.

(3) ***Results and Discussion on Task 3.4.3:*** Policy development

Results:

The project's initial policy session, "Regulatory and Policy Approaches for responding to ASF and Veterinary Transboundary Disease", was held at the 4th BTRP Regional One Health Research Symposium. This session took place during the first day of the concurrent One Health Science and Policy Forum on 21 May 2019. At the session, regulatory and policy aspects for responding to ASF and other animal diseases were discussed in addition to other UP-10-related issues. Participants defined key regulatory measures, which must be updated or changed in order to improve Ukraine's ASF crisis. These policy discussions and garnered insights were leveraged for planning the communication and outreach strategy pertinent to Goal 3 activities.

Unfortunately, the majority of the project's policy development objectives were to be completed during the 2020 One Health Science and Policy Forum at the 5th BTRP Regional One Health Research Symposium. However, due to the COVID-19

pandemic and resulting travel restrictions, these meetings were cancelled. As a result, Task 3.4.3 efforts were suspended and will constitute an essential component of future iterations of this body of work.

Discussion:
The following key policy points for consideration were identified by participants and were further elaborated on by project stakeholders via other policy-related meetings.

- Enhance slaughtering process: Slaughter only at designated slaughterhouses.
- Establish a compensation policy (for commercial farms and backyard farmers), with the assumption that more timely and improved compensation for stock loss would lead to better reporting.
- Implement livestock farm identification (to support traceability).
- Conduct further outreach activities: If farmers and hunters knew more about ASFV, would their behaviors and practices change and result in reduced ASFV spread?
- Provide recommendations for new policies that are practical, actionable, and can be evaluated for mitigating disease.
- Resolve gaps in the current veterinary biosurveillance system, engaging relevant legislative and regulatory authorities in solutions to overcome the challenges of previous reforms that created such gaps.

While the above initial requirements were highlighted by the project's participants and external stakeholders, the final process of bringing together the project's findings, previously identified policy concerns, and senior decisionmakers and elected officials was not realized due to work stoppage stemming from the COVID-19 pandemic. For example, though the groundwork was laid for inviting government officials and members of the Rada to the ASF Policy concurrent session at the 2020 BTRP Regional One Health Research Symposium, cancellation of the Symposium and enforcement of COVID-19 quarantine measures made it impossible to achieve completion of this Task. The project participants and international collaborators do hope that such efforts can be realized at a later date, especially considering the impact that UP-10 findings can have on conveying significant food safety gaps, which are detrimental to human and animal health.

Results and Discussion on Task 3.5: Produce a minimum of two, Ukrainian-recipient led, peer-reviewed publications on this work.

Results:
The following manuscripts are in preparation:
Manuscript title: ***Supporting control policies on African swine fever in Ukraine through a knowledge, attitudes and practice (KAP) survey targeting backyard farmers***.
Authors: Violeta Muñoz-Gómez, Oleksii Solodiankin, Nataliia Rudova, Anton Gerilovych, Serhiv Nychyk, Natalia Hudz, Tetiana Ukhovska, Mykola Syiuk, David Mustra, Marco De Nardi, Isabel Lechner, Manon Schuppers

Manuscript Title: **A Survey of Unlicensed Meat Markets in Ukraine to Evaluate Risks Related to the Spread of African Swine Fever Virus**.
Authors: Volodymyr Polishchuk, Mykola Sonko, Andrii Mezhenskyi, Maryna Sapachova, Mykola Sushko, Yevhen Tiniaiev, Iryna Khrystoieva, Oleksandr Kostiuk, Volodymyr Novosad, Andrii Rusyn, Yaroslav Riabets, Oleksandr Arnaut, Oleksandr Buhaichuk, Zinaiida Klestova, Oleksandr Napnenko, Oleksii Solodiankin, Nataliia Rudova, Anton Gerilovych, Serhii Nychyk, Nataliia Hudz, David Mustra, Karen Saylors, Mary Guttieri, Violeta Muñoz, Marco De Nardi, Manon Schuppers, Stephen Higgs, Craig Beardsley, Dana Vanlandingham

Potential manuscripts could also be prepared on the following topics:
- An overview of DTRA-supported programs to evaluate anthropogenic risk factors associated with the spread of ASFV in Ukraine.
- Approaches and methods for sample collection and the analysis of ASFV associated with non-commercial pork production in Ukraine.
- Routes of ASFV spread in Ukraine: An analysis of pork products from backyard farms and small-holdings in four oblasts.
- A review of ASF in Ukraine: 2012–2020.
- In-depth analysis on the findings of the UP-10 project including GIS data and review of biosafety and measures encountered at sales sites.
- Identification of critical Policy factors for Ukraine ASF Control.
- Creation of a publicly accessible UP-10 web site for Public Policy and Communications.

Discussion:
All project manuscripts that are in preparation have involved ongoing collaboration between Ukrainian scientists, administrative leadership, and SMEs. Regular meetings have fostered an open dialogue regarding current practices, policies, and gaps. The COVID-19 pandemic has necessitated an online, remote continuation of these conversations to continue data analysis for manuscript development.

2.6.2. Conclusion

The UP-10 project has shed light on several anthropogenic and socio-economic factors that are contributing to the spread of ASFV within Ukraine. One of the main goals of this project was to enlist people to collect data on the gray markets within the country. Teams were formed to collect samples in different Oblasts and were trained in the proper way to collect meat samples using protocols aimed at ensuring good biosafety and biosecurity measures were in place. The teams were also trained on how to inconspicuously collect other data that could be used to shed light on the socio-economic factors related to gray markets. Using a phone app developed for this project, the teams successfully collected detailed data on meat samples to be tested and on anthropogenic data, providing details on the purchase of the meat subsequently analyzed. These data can expand understanding of the relative importance of anthropogenic factors that contribute to the spread of ASF in Ukraine.

During February 2020 training, project participants listened to experiences of the various collection teams. Regional differences were identified in how the gray markets conduct business, including differences in ages of the participants, presence of PPE (e.g., gloves), and hygiene with regard to the handling of meat. Knowing regional differences is important for development of the type of messaging that might be effective in different areas within Ukraine. These discussions also helped to solidify the need to develop better control measures and implement policies that will lead to better biosecurity at farms and in the gray markets. Listening to the ideas and suggestions from various stakeholders gave insight into the complexity of this problem from an anthropogenic and socio-economic standpoint that would not be apparent from data itself.

Audience-appropriate materials were developed for the February 2020 workshop: Consumer Trade routes and Food Safety – Identifying and Reducing Risks for the Spread of Veterinary and other Food-borne Diseases of Concern. This included information of ASFV conveyed via lectures and discussions focused on the virus, pathogenesis in animals, historical perspective, transmission routes in different regions, and how eradication has been achieved in some areas. These were developed for a general audience. Emergency response training was also conducted to give an overview of issues that should be considered prior to an outbreak and to aid in discussions focused on how outbreaks are handled in Ukraine and if there should be any policy changes to better address an outbreak.

This project has identified areas that would benefit from further education, outreach, and policy changes. Results from the KAP survey suggested that comprehensive knowledge on ASF is not common amongst backyard farmers and that risky practices that influence the spread of ASF are regularly performed. These knowledge gaps are more evident in some Oblasts and exist despite the

implementation of various public outreach activities since the introduction of ASF into Ukraine in 2012. Backyard farms represent almost half of the pig production in Ukraine. Low biosecurity in backyard farms has been identified as playing a role in the spread and persistence of ASF in Eastern European countries, and the main risk factors for the spread of ASF virus among backyard farms include movement of infected pork meat, swill feeding, underreporting, and “emergency sales”. The KAP survey results confirmed the widespread presence of these risk factors in the Ukrainian backyard pig farming system. The main feeding practices of pigs mentioned by backyard farmers via UP-10 included their own production of pig feed (70.8%) and the use of kitchen leftovers (53.6%). One way to strengthen early detection of ASF in backyard holdings is through the supervision of home slaughtering by the veterinary services. A veterinarian is more likely to notice clinical symptoms of ASF in pigs presented for slaughter and may need to overcome fewer barriers to report ASF suspicions, which is the starting point for any official outbreak response measure. KAP survey results also showed that 92.9% of respondents carry out home slaughter and among them, only 14.8% do it with veterinary inspection. The outputs of this study will be leveraged for future public outreach activities and recommendations for policy-makers in Ukraine. An alternative policy would be to provide/require training on slaughter techniques to include disease recognition both in the live animal and in organs during butchering.

The work conducted by the UP-10 project has, for the first-time, provided molecular diagnostics confirmation that ASFV-contaminated meat products are in circulation within Ukraine and can be procured from illegal vendors. These efforts represented a qualitative, not quantitative, survey, and though the number of positive samples was limited, these findings raise widespread concern of the potential for ASFV transmission via the illegal trade network. Thus, enhanced understanding and acceptance of the relevance of the study’s qualitative results is imperative. This study also points to the need for more expansive follow-on studies that help identify how pervasive contaminated products are within the illegal trade networks, with an eye on quantifying the potential for the translocation of contaminants and introduction/spread of diseases of concern. Through such efforts, gaps in the biosurveillance network that permit the spread of disease, via illegal sales and other anthropogenic factors, can be identified and overcome.

Although this research project focused on ASFV, the methodologies that were developed, the relationships that were established, and the data that were collected may have applications to other transboundary animal diseases. Proactive evidence-based/data-driven policies that identify a pathogen soon after introduction provide the best approach to controlling the spread of the pathogen and enable eradication. Other pathogens, such as foot and mouth

diseases (FMD), could be as devastating to Ukraine as ASFV. The DTRA-funded research on ASFV could be extended to be more generic but highly impactful. The strong inter-stakeholder and inter-agency cooperation realized through the CBR project UP-10 has demonstrated the capacity for future cooperation to reduce inefficiencies and navigate around bureaucratic barriers that have impeded prior efforts to improve the effectiveness of the biosurveillance system and reduce the spread of pathogens of concern to the veterinary and animal husbandry communities. This was demonstrated through the fact that all the data within the Project was coordinated by NULES and the laboratory tests were conducted jointly with the two NAAS institutes. In addition, all the project's face-to-face meetings and workshops gathered together both senior regulatory officials, biosurveillance system scientists, and other concerned stakeholders, which further contributed to the project's ability to avoid bureaucratic procedures and find agreement quickly on key scientific objectives. Everything possible should be done to retain and expand these relationships, so that Ukraine and other countries in the region are better prepared when the next pathogen emerges. The UP-10 project clearly demonstrated the capabilities and resourcefulness of Ukrainian scientists and other workers. The team utilized equipment provided by DTRA, which was used to great effect for sample analysis. A potential limitation was the lack of facilities approved to handle infectious ASFV. The development of such facilities and training of personnel to work in high containment could be an area for future funding. Development of a self-sustaining regional network of experts from multiple countries could provide early warnings of pathogen introduction that would enable rapid response.

The availability of such facilities would also allow experiments with ASFV. For example, although positive samples were detected, the relationship between infection and detection remains unknown. Sensitivity of the PCR-based detection could, for example, be influenced by the type of product. Was for example detection of ASFV in salo but not in blood sausage dues to a higher level (titer) of ASFV in salo? Were negative results from blood sausage due to a lack of infection or due to PCR failure due to product characteristics? Being able to perform experiments with ASFV-spiked products at a known titer, with serial dilutions, would provide this understanding and could provide food-specific optimized protocols. If containment facilities were not approved for such work in Ukraine, samples could be produced in the laboratories of collaborators.

With regard to ASFV in Ukraine, questions remain, requiring enhanced understanding of wild boar populations and their interactions with domestic pigs. The unlicensed market survey was successful but, due to limited time and funding, provides only a snapshot of the situation. With the methods and personnel now established, an extension of this study throughout 12 months, or ideally over 2 full-years, could identify patterns associated with ASFV-positive animals raised in backyard farms. The identification of positive samples proved

the detection capabilities, but it could be that at times when samples were not collected, the incidence could be higher and have a greater impact on ASFV spread between regions. All samples that were collected were said to be home produced; however, in discussions with stakeholders, it was mentioned that some pork products are imported for resale. The extent of this practice was not revealed by the survey.

2.6.3. Issues or Concerns

The COVID-19 pandemic disrupted a few activities. For example, bi-weekly meetings between Ukraine-based participants and SMEs were postponed due to reprioritization of work responsibilities, which thereby limited discussions concerning data intended for publication and policy development. To address these limitations, work arounds were adopted; e.g., remote training was implemented, and materials were audio-recorded and shared with participants. In addition to COVID-related concerns, SMEs noted that expanded mentoring and training are necessary for effective development, validation, and troubleshooting of research methodology. When planning/conducting research, greater emphasis should be placed on face-to-face interactions between SMEs and project participants to overcome cultural norms that favor rote processes, which limit the analytical perspective necessary to successfully run experiments.

2.6.4. Selected References

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Further Reading from OIE and FAO Protocols:

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Appendix A. UP-10 Funding Report

Approved Budget: $1,011,875.23

Costs to Date (30 June 2020, Direct Costs): $592,756.49

Issues or Concerns: Final billing in underway and will be reflected in future invoicing.

Task Name	B&V Direct Cost	B&V Total Cost
Veterinary CBR Project UP-10 Implementation – Approved Budget	$971,031.35	$1,011,875.23
Veterinary CBR Project UP-10 Implementation – Cost to Date (30 June 2020)	$592,756.49	$657,987.26

Appendix B. UP-10 Impact Table

Activity	Task/Team	Key Accomplishments
Define geographical and environmental factors associated with establishment and spread of ASFV through wild boar movements	Tasks 1.1, 1.2	• The geographical distribution of ASF cases and the seasonality of ASF (as mapped/demonstrated by FSCP and utilized to select the project's primary sites and time of sample collection) were determined to be predictive of the epidemiological situation of ASF in Ukraine and yielded positive market samples. These findings emphasize the importance of FSCP's continued collection and analysis of ASF data.
UP-10 Pork Product Sample Collection	Tasks 2.1, 2.2	• The significant capacity of regional FSCP offices to contribute to larger research programs and to implement protocols for scientific studies was demonstrated. • Confidence was increased regarding bio-secure handling of meat samples for laboratory testing when collected in rural environments outside of routine surveillance activities. • Very interactive discussions on approaches brought together professionals from different Oblasts, thereby ensuring consistency and offering opportunity to share experiences.
Laboratory Investigations	Tasks 2.1, 2.2	• Investigations indicated circulation of ASFV in multiple regions of Ukraine within illegally sold pork products. • Sivital's ASFV test kit (Vitebsk, Belarus) was validated, with all results 100% aligned with the only current Ukraine approved-for-use test system, LSI. By expanding the field of government-approved test systems, routine operating costs for SSRILDVSE can be reduced, and through regional production, procurement times can be shortened.

Activity	Task/Team	Key Accomplishments
Increasing regional collaboration and stakeholder integration	Workshops and Meetings	• Interactive discussions engaged producers and regulators, highlighting challenges and helping to identify solutions that better integrate their perspectives. • The strong inter-stakeholder and inter-agency cooperation realized through the project demonstrated the capacity for future cooperation to reduce inefficiencies and navigate around bureaucratic barriers, which have impeded prior efforts to improve the effectiveness of Ukraine's biosurveillance system. • Through multi-regional discussions concerning project activities that required regional representatives directly engage with the public, greater awareness and insight were acquired by FSCP representatives, which highlighted significant disconnects between stakeholder/public perspectives and FSCP assumptions and perspectives.
Capacity Building for computational methodologies	Tasks 1.1, 1.2, 3.1, 3.2 Task 3.2- Develop training curricula for GIS and perform outreach to inform local, regional and national policy development (SAFOSO)	• The first GIS training event enhanced skills on spatial analysis and the use of GIS software. • The second GIS training event fostered skills on interpreting spatial analysis and GIS outputs under disease control scenarios. • The Fellowship Program entitled "Spatial Modeling and R Programming Training", which was hosted by the Spatial Epidemiology & Ecology Research Laboratory (SEER Lab) at the University of Florida (Gainesville, FL, USA), graduated a senior T3 trainer for BTRP Ukraine.
Track Anthropogenic and Socio-Economic Factors	Tasks 2.3, 2.4	• Awareness was raised regarding the potential for anthropogenic spread and the national scope of this risk.

Activity	Task/Team	Key Accomplishments
		• The project demonstrated that ASFV is present in multiple pork products and in food products, which visually did not appear to pose a threat to human or animal health. • Through sales encounters and other surveys, the project demonstrated the economic drivers that sustain illegal sales and the willingness to violate regulatory requirements and other laws.
Public outreach activities and policy development	Task 3.4- Educate and perform outreach to inform local, regional, and national policy development (SAFOSO/Metabiota)	• A communication plan was developed concerning ASF as part of a national outreach strategy. • Knowledge gaps and regular risk practices were identified concerning backyard farmers and wild boar hunters. • Scientific manuscripts are in preparation based on the results of surveys administered to backyard farmers. • Key policy aspects regarding the country's current regulatory and policy approach on ASF were identified and discussed. • Key stakeholders and partners for future policy development and advocacy coalitions were identified. • KAP survey methodology was directly institutionalized as a part of veterinary graduate education by NULES.
Workshops and Scientific Advancement of Capacity	Workshops and Conferences	• Presentations of UP-10 data in workshops and international meetings were well received, including at the 2020 ASM Biothreats meeting and DTRA's 2019 Science Program Review. • The UP-10 project team has been invited to present the project's novel findings at the FAO meeting "African swine fever unprecedented global threat: A challenge to food security, wildlife management, and conservation" (Sept. 2020; Warsaw, Poland).

Appendix C. Updated Work Plan

Updates to the Work Plan were included to Quarterly Factsheets

UP-10: Regional field-to-table risk assessment of the spread of African swine fever virus (ASFV) across Ukraine in wild fauna and via consumer trade routes – insight into the development of effective ASFV quarantine strategies and public policy

	Activities	Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
KOM	Kickoff meeting	Metabiota	SAFOSO, KSU, all															
GOAL 1	**DEFINE GEOGRAPHICAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH ESTABLISHMENT AND SPREAD OF ASFV THROUGH WILD BOAR MOVEMENTS**																	
Task 1.1	**Perform spatial modeling of existing data on wild boar occurrence, habitat landscape structure, and seasonal movement across Ukraine**	SSUFSCP/NULES																
a	Development of database to gather historical data on the density of pig farming in Ukraine	SSUFSCP	SSRILDVSE/IECVM /IVM/Others															
b	Collection and analysis of historical data on the density of pig farming in Ukraine	SSUFSCP	SSRILDVSE/IECVM /IVM/Others															
c	Development of database on wild boar populations, geographic locations, and movement in Ukraine	SSUFSCP/SFRA	SSRILDVSE/IECVM /IVM/Others															
d	Collection and analysis of historical data on the abundance of wild boar population and host habitat distribution and hunting activity in Ukraine	SSUFSCP/SFRA	SSRILDVSE/IECVM /IVM/Others															
e	Collection and analysis of multi-annual observational data of the disease in Ukraine	SSUFSCP	SSRILDVSE/IECVM /IVM/Others															
f	Perform Spatial modeling of disease risk in feral swine and the interface with disease perpetuation in domestic population to test alternative quarantine strategies	UFZ/UoF/IVM	SSRILDVSE/IECVM /IVM/SSCIBSM/Others															
Task 1.2	**Support capacity building for spatially explicit computational methodologies within the participating Ukrainian organizations.**	UoF/IVM																
a	Development of training materials on computational modeling	IVM	UFZ/UoF/Metabiota/NULES/SSRILDVSE/IECVM/IVM/SSCIBSM/Others															



	Activities	Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
b	Training 1 on Computational Modeling in collaboration with subject matter experts	IVM	UFZ/UoF/NULES/ SSRILDVSE/IECVM /IVM/SSCIBSM/ Others															
c	Training 2 on Computational Modeling in collaboration with subject matter experts	IVM	UFZ/UoF/NULES/ SSRILDVSE/IECVM /IVM/SSCIBSM/ Others															
d (see task 3.1)	Establish and implement a GIS and Computational Modeling Fellowship (see task 3.1)	UFZ/UoF																
GOAL 2	**TRACK ANTHROPOGENIC AND SOCIO-ECONOMIC FACTORS**																	
Task 2.1	**Ensure proper protocol and biosecurity throughout sample collection, shipping, and testing.**	**KSU**																
a	Thorough review of existing protocols for Goal 2 to determine effectiveness and clarity	SSRILDVSE	KSU/Metabiota															
b	Development/update of SOPs	SSRILDVSE	KSU/Metabiota															
c	Communication outreach to research team members to ensure the protocols, biosecurity measures, and SOP's are understood and followed	SSRILDVSE	KSU/Metabiota															
Task 2.2	**Analysis of biological samples of pork products from various stakeholders to test for ASF.**	**SSRILDVSE**																
a	Design of survey for biological sample collection from various stakeholders																	
b (former task 2.2)	Collect biological samples of pork products	SSRILDVSE/KSU/ BV-Labyrinth/	SSUFSCP/IECVM/I VM/Metabiota/ Others															
c (former task 2.3)	Conduct laboratory investigations of collected specimens using PCR/RT-PCR assays to determine presence of ASFV	SSRILDVSE/KSU	SSUFSCP/IECVM/I VM/Others															
e (former task 2.4)	Analysis of results	SSRILDVSE/KSU	SAFOSO/ Metabiota/BV-Labyrinth/IECVM/ IVM															




	Activities	Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
Task 2.3 (former task 2.5)	**Demonstrate and document anthropogenic factors contributing to the spread of ASF in Ukraine and the need to implement effective biosecurity and control measures for preventing farm-to-farm and farm-to-wildlife spread.**	KSU/NULES																
a	Design of anthropogenic factors survey (i.e. case control study) including assessment of biosecurity measures and anthropogenic and socio-economic factors associated to spread of ASF	SSUFSCP/	SSRILDVSE/IECVM /IVM/Others															
b	Field visit to assess biosecurity measures and anthropogenic and socio-economic factors	SSRILDVSE/KSU	BV-Labyrinth/ Metabiota															
c	Analysis of results	SSRILDVSE/KSU	SAFOSO/ Metabiota/BV-Labyrinth															
Task 2.4	**Assess the potential risk of ASFV spread within Ukraine and across regional borders via commercial trade routes of pigs and pig products, the illegal distribution and transport of pigs and pig products, and wild boar movements.**																	
a	Under discussion	SSUFSCP/BV-Labyrinth/KSU	SAFOSO IVM/ SSRILDVSE/IECVM /Others															
b	Under discussion	TBD																
c	Under discussion	TBD																
GOAL 3	**PUBLIC POLICY AND COMMUNICATION THROUGH TRAINING, EDUCATION, AND OUTREACH**																	
Task 3.1	**Establish a GIS and Computational short-term Modeling Fellowship.**	BV-Labyrinth																
a	Collection of nomination for the Fellowship	UFZ/Metabiota /UoF																
b	Evaluation and final selection of the Fellowship candidate	UFZ/Metabiota /UoF																
c	Implementation of short-term Fellowship at University of Florida (UoF)	UoF/BV-Labyrinth	UFZ/ BV-Labyrinth															
Task 3.2	**Develop training curricula for GIS and perform outreach to inform local, regional, and national policy development.**	IVM (M. Bezimennyi)																
a	Development of training materials on GIS and spatial analysis	SAFOSO	UFZ/UoF/IVM/ Metabiota/BV-Labyrinth															

Activities		Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
b	Training 1 on GIS in collaboration with subject matter experts	SAFOSO	UFZ/UoF/NULES/ SSRILDVSE/IECVM /IVM/SSCIBSM/ Others															
c	Training 2 on GIS in collaboration with subject matter experts	SAFOSO	UFZ/UoF/NULES/ SSRILDVSE/IECVM /IVM/SSCIBSM/ Others															
Task 3.3	**Develop audience-appropriate materials to support education and public outreach strategies.**	IVM/NULES																
a (see 1.2.a)	Development of training materials on computational modeling (see 1.2.a)	IVM/UFZ/UoF	Metabiota															
b (see 3.2.a)	Development of training materials on GIS and Spatial analysis (see 3.2.a)	IVM/UFZ/UoF	SAFOSO/ Metabiota															
c	Evaluation of existing outreach materials by members of the outreach group	SAFOSO	Metabiota/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
d	Outreach materials: Educational material on biosecurity for Officials and Regional Veterinarians	SAFOSO	Metabiota/BV-Labyrynth/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
e	Outreach materials: Communication and awareness materials (e.g., brochures, videos, posters)	SAFOSO	Metabiota/BV-Labyrinth/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
Task 3.4	**Educate and perform outreach to inform local, regional, and national policy development.**	SSUFSCP/NULES																
Task 3.4.1	**Public outreach**	NULES																
a	Establish Public Outreach Working Group	SAFOSO	Metabiota/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															

	Activities	Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
b	Preparation of face-to-face workshop, Public Outreach Working Group	SAFOSO	Metabiota/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
c	Delivery of face-to-face **workshop**, Public Outreach Working Group: Development of a national outreach strategy	SAFOSO	Metabiota/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
d	Revision of national outreach strategy following KAP study through 2 videoconferences	SAFOSO	Metabiota/BV-Labyrinth/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
e	Implementation of outreach activities	SAFOSO / NULES																
Task 3.4.2	**KAP survey**	NULES (V. Polishchuk)																
a	Design KAP-survey	SAFOSO	Metabiota/BV-Labyrinth/IVM /SSRILDVSE/ IECVM/SSCIBSM/ Others															
b	Preparation KAP survey through video conferences (including preparation of data collectors)	SAFOSO																
c	Pilot-testing of KAP-intervention	SAFOSO																
d	KAP-intervention implementation																	
e	Analysis of KAP-results	SAFOSO																
f	Preparation of face-to-face workshop, KAP results	SAFOSO																
g	Implementation of Face-to-face **workshop**: KAP results	SAFOSO																
Task 3.4.3	**Policy development**	BV-Labyrinth/ NULES																
a	Establish Policy Development Working Group	BV-Labyrinth	Metabiota/ BV-Labyrinth SAFOSO/IECVM /IVM/SSRILDVSE /SSCIBSM/Others															

	Activities	Task leader	Collaborators	Timeline														
				Q 1			Q 2			Q 3			Q 4			Q 5		
				Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20
b	**1st meeting** of the Policy Development Working Group during 2019 BTRP-UA Regional One Health Symposium, Kyiv	BV-Labyrinth	SAFOSO/ Metabiota															
c	Preparation of face-to-face workshop, Policy Development Working Group	SAFOSO/ /BV-Labyrinth	Metabiota															
d	Delivery of face-to-face **workshop**, Policy Development Working Group	SAFOSO/ /BV-Labyrinth	Metabiota															
e	Video conferences: Policy Development Working Group	SAFOSO/ BV-Labyrinth																
f	**Final meeting** of the Policy Development Working Group	BV-Labyrinth /NULES	SAFOSO															
Task 3.5	Produce a minimum of two, Ukrainian-recipient led, peer-reviewed publications on this work.	BV-Labyrinth																
a	Preparation of KAP manuscript		SAFOSO															
b	Preparation of anthropogenic risk factors paper		Metabiota/BV-Labyrinth															
c	Preparation of computational modelling paper		UoF/UFZ															
d	Preparation of biosurveillance/market data manuscript		KSU															

Appendix D: KAP Questionnaire for Backyard Farmers

Knowledge Attitudes and Practices (KAP) Survey- ASF in Ukraine

Questionnaire 1

Target group: Small holders-backyard farms

SECTION 1: DEMOGRAPHIC INFORMATION

1. Give the name of the Oblast, the Rayon and the Village where you live

   Oblast ______________
   Rayon ______________
   Village ______________

2. Who is the main person taking care of the pigs? Select one answer.
   - ☐ I am the main person taking care of the pigs.
   - ☐ Another member of the household living on the premise.
   - ☐ External person to the household.
   - ☐ Both (member of the household and an external person).

3. How old is the main person involved in taking care of the pigs? Select one answer.
   - ☐ ≤20 years old.
   - ☐ 21-35 years old.
   - ☐ 36-50 years old.
   - ☐ 51-65 years old.
   - ☐ ≥66 years old.

4. What is the highest educational level of the main person involved in taking care of the pigs? Select one answer.
   - ☐ Primary school.
   - ☐ Secondary school.
   - ☐ Vocational training.
   - ☐ Technical studies.
   - ☐ University.
   - ☐ Post-graduate.

5. Are any of these activities carried out by any of the members of your household? Select all that apply.
   - ☐ Work in the slaughterhouse.
   - ☐ Hunting.
   - ☐ Work in a pig farm.
   - ☐ Work in a catering facility (e.g. waiter, cook).
   - ☐ Work in the forest (e.g. forest-guard).
   - ☐ No member of the household carries out any of the aforementioned activities.

- ☐ Offer slaughter and butchery services to the private sector.

SECTION 2: BACKYARD FARM CHARACTERISTICS

6. How many pigs do you have in your backyard? Select one answer.
   - ☐ 1
   - ☐ 2-3
   - ☐ 4-5
   - ☐ ≥6

7. What kinds of pigs predominate in your backyard? Select the closest option.
   - ☐ Sows.
   - ☐ Boars.
   - ☐ Fattening pigs.

8. Do you have a pen for the pigs? Select one answer.
   - ☐ Yes
   - ☐ No

9. Can your pigs move around freely out of the pen? Select one answer.
   - ☐ Yes, but only on my own premise.
   - ☐ Yes, they can go outside of their my own premise.
   - ☐ No, they cannot go outside their own premise.

10. How many people, in total (i.e. from the household and outside the household), normally interact with the pigs? Select one answer.
    - ☐ 1
    - ☐ 2-3
    - ☐ 4-5
    - ☐ ≥6

11. How many times in total per day do these people interact with the pigs? Select one answer.
    - ☐ <1
    - ☐ 1
    - ☐ 2
    - ☐ 3
    - ☐ 4
    - ☐ >4

12. Which of these statements are the closest to the feeding habits of your pigs? Select all that apply.
    - ☐ I use commercial feed for pigs.
    - ☐ I use commercial animal feed but not exclusively for pigs.
    - ☐ I use kitchen leftovers.
    - ☐ I produce my own pig feed.
    - ☐ I share the commercial feed for pigs with my neighbours.
    - ☐ My neighbour and I share the kitchen leftovers to feed our pigs.
    - ☐ Other:_________.

13. How are your pigs slaughtered? Select one option.
    - ☐ Home slaughter without veterinary inspection.
    - ☐ Home slaughter with veterinary inspection.
    - ☐ At the slaughterhouse.

14. What do you normally do with the edible food products after slaughtering the pigs? Select all that apply
    - ☐ Consumption within my household.
    - ☐ Give it to neighbours/relatives.
    - ☐ Sell it to the local butchery.
    - ☐ Sell it to the market in my village or in a neighbouring village.
    - ☐ Sell it to traders.
    - ☐ Other:_________.

15. What do you normally do with the non-edible food products after slaughtering the pigs? Select all that apply.
    - ☐ Sell them to a third party.
    - ☐ Use them as feed.
    - ☐ Dispose of them in a composting pit (pile) together with manure for further use as an organic fertilizer.
    - ☐ Dispose of them at official dump.
    - ☐ Dispose of them informally (e.g. forest, daily rubbish bin).
    - ☐ Other:_________.

SECTION 3: KNOWLEDGE OF AFRICAN SWINE FEVER (ASF) IN UKRAINE

16. Select one answer for each of the following questions:

▪ Have you ever heard about African Swine Fever (ASF)?	☐ Yes	☐ No
▪ Do you know anybody who has experienced either a suspected or a confirmed case of ASF in his/her pigs?	☐ Yes	☐ No
▪ Do you feel you are well-informed about how ASF can be transmitted?	☐ Yes	☐ No
▪ Do you feel confident that you can recognise the clinical signs of ASF?	☐ Yes	☐ No

17. Which of the following clinical signs do you associate with ASF in pigs? Select all that apply.
    - ☐ Fever
    - ☐ Diarrhoea
    - ☐ Vesicles around the tongue and lips
    - ☐ Lameness
    - ☐ Reddening or darkening of the skin
    - ☐ High mortality
    - ☐ Vomiting
    - ☐ Lethargy
    - ☐ Difficulty to breathing
    - ☐ Stillborn or weak piglets
    - ☐ Nervous signs
    - ☐ I do not know any signs

18. Through which of the following pathways can pigs get infected with ASF? Select all that apply.
    - ☐ Through direct contact with a diseased pig or carcass of a diseased pig.
    - ☐ Through direct contact with diseased wild boars or carcass of a diseased wild boar.
    - ☐ Through consumption of kitchen waste.
    - ☐ Through consumption of leftovers from the slaughter process.
    - ☐ Through contact with contaminated manure.
    - ☐ Through contact with contaminated clothing, footwear and/or transport vehicles.
    - ☐ Through a bite of an infected tick.
    - ☐ Through use of contaminated surgical equipment.
    - ☐ Through sexual contact.
    - ☐ Through airborne transmission.
    - ☐ I do not know any pathways.

19. Which preventive measures do you take to protect your pigs against ASF? Select all that apply.
    - ☐ No exchange of feed or bedding with other backyards.
    - ☐ Quarantine period for new animals in a separate room.
    - ☐ Provision of a salt block.
    - ☐ Vaccination.
    - ☐ No introduction of pigs from non-commercial farms.
    - ☐ My pigs are not allowed to roam around freely outside of my premise.
    - ☐ Using only commercial pig feed.
    - ☐ Disinfecting and cleaning the areas around the backyard.
    - ☐ My entire premise is fenced.
    - ☐ I do not take any measures.
    - ☐ Other:___________.

SECTION 4: SUSPICION OF AN ASF CASE

20. Have you ever suspected that you may have ASF in your pigs? Select one answer.
    - ☐ Yes
    - ☐ No

21. What can be the reasons that might lead you to **not** suspect ASF in your backyard pigs? Select all answers that cover you opinion best.
    - ☐ My oblast is free of ASF.
    - ☐ I'm not sure if I would be able to recognise the clinical signs of ASF in my pigs.
    - ☐ My pigs are healthy.
    - ☐ I follow a strict procedure for the destruction of carcasses from diseased pigs.
    - ☐ The pigs of my neighbours are healthy so I do not have any cause for concern.
    - ☐ I take care of the food stuff of my pigs.
    - ☐ I only buy new pigs from places that I know and trust.
    - ☐ I vaccinate my pigs.
    - ☐ There are no wild boars in my area.
    - ☐ I do suspect ASF in my pigs.
    - ☐ Other:__________.

22. What would you do if you suspect ASF in your pigs? Select all answers that are closest to your reaction.
    - ☐ Slaughter the animal and use the meat for consumption at home.
    - ☐ Slaughter the animal and sell the meat via the local market.
    - ☐ Kill the animal and dispose of the carcass.
    - ☐ Sell the sick animal.
    - ☐ Sell the remaining animals to not lose more money.
    - ☐ Report the suspicion to the veterinarian.
    - ☐ I do not do anything out of my ordinary routine.
    - ☐ Other:________.

23. When you find that one of your pigs is ill, how long do you wait until you take a first action? Select the closest answer to your reaction.
    - ☐ I do not wait at all- I look for help immediately.
    - ☐ I usually wait 1 day, if the pig is still ill the next day, then I take action [Go to question 24.
    - ☐ I usually wait a few days to see if the pig recovers. If not, then I take action [Go to question 24].
    - ☐ I do not take any action. Either the pig recovers by itself, or it dies.

24. According to your previous answer, you prefer waiting some time and to see if the pig recovers by itself before taking action. Would you take action earlier if more pigs start getting ill during that waiting period? Select the closest answer to your reaction.
    - ☐ Yes, as soon as I see that one additional pig starts getting ill, I would take action.
    - ☐ Yes, as soon as I see that two or more additional pigs are getting ill, I would take action.
    - ☐ No, I would still wait a bit longer to see if the pigs recover by themselves.

25. What would you do if you hear that pigs in neighbouring settlements are dying? Select one option.
   - ☐ I would not do anything.
   - ☐ I would ask the veterinarian I know for more information.
   - ☐ I would pay much more attention to my pigs.
   - ☐ I would inform the veterinary service about what I heard.
   - ☐ I would search more information through social media (e.g. internet).
   - ☐ Other:_____.

SECTION 5: REPORTING OF AN ASF CASE

26. Have you ever reported a suspected case of ASF on your premises? Select one answer.
   - ☐ Yes
   - ☐ No
27. What are reasons that would **prevent you from reporting** an ASF suspicion? Select all answers that apply.
   - ☐ I do not know how to report.
   - ☐ If I report it, the authorities will kill my pig.
   - ☐ If I report it, it will take ages until I get the financial compensation.
   - ☐ I'm not able to recognise a suspected case of ASF in the pigs.
   - ☐ It would negatively affect the sales of my pigs negatively.
   - ☐ My neighbours would not appreciate it when I report an ASF suspicion.
   - ☐ I always report it.
   - ☐ I have never suspected an ASF case.
   - ☐ Other:__________.

28. How would you feel if you reported an ASF-suspicion in your pigs to the authorities that later turned out to be non-infected? Select the closest answer.
   - ☐ Ashamed, my neighbours will make fun of me. I expected a positive result
   - ☐ Very happy of not having ASF in my pigs
   - ☐ Good, I do not care what the test result is, I followed the rules
   - ☐ Discouraged, I have wasted lost my time and energy
   - ☐ Relieved, during the waiting period I was very worried
   - ☐ Other:_________

29. What would be the most convenient way for you to officially report an ASF-suspicion? Select one answer.
   - ☐ Via phone call to a known veterinarian
   - ☐ Via a phone call to a "hotline" of the veterinary service
   - ☐ Via internet through a special website of the veterinary service
   - ☐ Other:______

30. What is your opinion about the Official State Veterinary Service? Please indicate your opinion with an "X" for each of the following criteria.

Criteria:	Fully disagree	Disagree	Slightly disagree	Slightly agree	Agree	Fully agree
Competent						
Reliable						
Efficient						
Trustworthy						
Responsive in timely manner						
Easily accessible						

ONE LAST QUESTION

31. How would you grade your honesty in your answers? (5 means perfectly honest)

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Thank you very much for your collaboration.

Kind Regards,

The UP-10 Project

Appendix E: KAP Questionnaire for Wild Boar Hunters

Knowledge Attitudes and Practices (KAP) Survey- ASF in Ukraine
Questionnaire 2

Target group: Wild boar hunters

SECTION 1: DEMOGRAPHIC AND PROFILE INFORMATION

1. Give the name of the Oblast, the Rayon and the Village where you **live**.
   Oblast _____________
   Rayon _____________
   Village _____________

2. How old are you? Select one answer.
   - ☐ ≤20 years old.
   - ☐ 21-35 years old.
   - ☐ 36-50 years old.
   - ☐ 51-65 years old.
   - ☐ ≥66 years old.

3. What is the highest education level of the main person involved in taking care of the pigs? Select one answer.
   - ☐ Primary school
   - ☐ Secondary school
   - ☐ Vocational training
   - ☐ Technical studies
   - ☐ University
   - ☐ Post-graduate

4. Do you typically hunt in the same Rayon as where you live? Select one answer.
   - ☐ Yes
   - ☐ No

5. How often do you go hunting wild boars during the hunting period in Ukraine? Select one answer.
   - ☐ I go hunting at least once.
   - ☐ I go hunting between 2-3 times.
   - ☐ I go hunting at least 4 times.
   - ☐ It has been a few years since I have not gone hunting.
   - ☐ I sometimes go hunting outside the official hunting period.

6. How often do you hunt in Ukraine outside the hunting period? Select one answer.
   - ☐ I go hunting at least once.
   - ☐ I go hunting between 2-3 times.
   - ☐ I go hunting at least 4 times.
   - ☐ It has been a few years since I have not gone hunting.

7. Select one answer for each of the following statements:
   - Do you have a pig backyard farm at home? ☐ Yes ☐ No
   - Do you work in a pig farm? ☐ Yes ☐ No
   - Do you work in a slaughterhouse? ☐ Yes ☐ No
   - Do you work in the forest? (e.g. forest-guard) ☐ Yes ☐ No
   - Do you work in catering (e.g. waiter, cook) ☐ Yes ☐ No
   - Do you offer slaughter and butchery services to the private sector? ☐ Yes ☐ No

8. When you go hunting and bring your own food supplies for the day, what would you do with leftover food? Select one answer.
   - ☐ I typically throw it away in the environment.
   - ☐ I carry it back home.
   - ☐ I typically throw it away at an official disposal location.
   - ☐ Other:_____________.

9. What do you normally do with the wild boars that you have hunted? Select all that apply.
   - ☐ I use the meat for home consumption.
   - ☐ I give the meat to neighbours/relatives/friends.
   - ☐ I sell it on the local market.
   - ☐ I sell it to a trader.
   - ☐ I do not use it at all.
   - ☐ I leave it at the shooting site (forest) as feed for predatory animals.
   - ☐ I leave it at a special site in the forest as bait for shooting other predatory animals.
   - ☐ I leave it in a closed waste pit in the forest.
   - ☐ I bring the whole carcass to an equipped point for dressing and further processing.
   - ☐ This is not my business, I do not think about it.
   - ☐ Other:__________.

10. Do you normally receive notifications about the appearance or spread of wildlife diseases? Select one option.
    - ☐ Yes, always.
    - ☐ Yes, sometimes.
    - ☐ No.

SECTION 2: KNOWLEDGE OF AFRICAN SWINE FEVER (ASF) IN UKRAINE

11. Select one answer for each of the following questions:
    - Have you received any training or information about wild boar diseases? ☐ Yes ☐ No
    - Have you ever heard about African Swine Fever (ASF)? ☐ Yes ☐ No
    - Do you feel you are well-informed about how ASF can be transmitted? ☐ Yes ☐ No
    - Do you feel confident that you can recognise the clinical signs of ASF? ☐ Yes ☐ No
    - Do you know anybody who experienced either a suspected or confirmed cases of ASF? ☐ Yes ☐ No
    - Do you think that ASF is an animal health problem in Ukraine? ☐ Yes ☐ No

12. Which of the following clinical signs do you associate with ASF in wild boar? Select all that apply.
    - ☐ Fever
    - ☐ Diarrhoea
    - ☐ Vesicles around the tongue and lips
    - ☐ Lameness
    - ☐ Reddening or darkening of the skin
    - ☐ High mortality
    - ☐ Vomiting
    - ☐ Lethargy
    - ☐ Difficult to breath
    - ☐ Stillborn or weak piglets
    - ☐ Nervous signs
    - ☐ I do not know any signs

13. Through which of the following pathways can wild boars get infected with ASF? Select all that apply.
    - ☐ Through direct contact with a diseased pig or carcass of a diseased pig.
    - ☐ Through direct contact with diseased wild boars or carcass of a diseased wild boar.
    - ☐ Through consumption of kitchen waste.
    - ☐ Through consumption of left-overs from the slaughter process.
    - ☐ Through contact with contaminated manure.
    - ☐ Through contact with contaminated clothing, footwear and/or transport vehicles.
    - ☐ Through a bite of an infected tick.
    - ☐ Through sexual contact.
    - ☐ Through air-borne transmission.
    - ☐ I do not know any pathways.

14. How do you think the wild boar population has changed in the region where you have hunted over the past 3-5 years?
    - ☐ Increased (Go to Question 16).
    - ☐ Decreased (Go to Question 15).
    - ☐ Remained at the same level (Go to Question 16).

15. What do you consider to be the most important reason for the reduction in the number of wild boar in the area where you are hunting? Select one option.
    - ☐ Excessive shooting during hunting.
    - ☐ Poaching
    - ☐ Deaths from ASF.
    - ☐ Migration to neighbouring lands with the best feeding base.
    - ☐ Conducting an unlimited “sanitary” killing for disease control reasons.
    - ☐ Migration to neighbouring lands in response to the sanitary killing.
    - ☐ Other:____________.

SECTION 3: SUSPICION OF AN ASF CASE

16. Have you ever suspected ASF in a sick or dead wild boar when you are hunting? Select one answer.
    - ☐ Yes
    - ☐ No

17. What would be the reasons that might lead you to **not** suspect ASF in wild boars when you are hunting? Select all answers that cover your opinion best.
    - ☐ The oblast in which I hunt is free of ASF.
    - ☐ I have never seen a sick or a dead wild boar.
    - ☐ I'm not sure if I would be able to recognise the clinical signs in a wild boar.
    - ☐ I have never thought about it.
    - ☐ I always suspect ASF in wild boars.
    - ☐ Other:___________.

18. What would you do if you suspect ASF in wild boars when you are hunting? Select the closest answer.
    - ☐ Report it to the authorities.
    - ☐ I would just leave the carcass where I shot it.
    - ☐ I would take some pieces of meat that do not seem to be affected and leave the rest where I shot it.
    - ☐ I would take the entire carcass and dispose of it at an official dump site.
    - ☐ I would take the carcass to the local veterinary authority for investigation.
    - ☐ I would ask someone for help to report it to the authorities.
    - ☐ Other:____________.

19. Why would you think that it is important that hunters report suspicious cases of ASF to the authorities? Select the closest answer.
    - ☐ I do not think it is important.
    - ☐ Hunters have a critical role in detecting diseases in wild animals.
    - ☐ Only hunters hunting near the borders have an important role in reporting suspected cases.
    - ☐ When hunters report disease in wild boar, they contribute to fighting disease in domestic pigs as well.
    - ☐ Other:__________.

SECTION 4: REPORTING OF AN ASF CASE

20. Have you ever reported a suspected case of ASF? Select one answer.
    - ☐ Yes
    - ☐ No

21. What are the reasons that would **prevent you from reporting** an ASF suspicion? Select all answers that apply.
    - ☐ My hunting colleagues would not appreciate it.
    - ☐ I do not want to waste hunting time by dealing with the authorities.
    - ☐ The authorities will take the wild boar carcass.
    - ☐ If I report it, it will take ages until I get the financial compensation.
    - ☐ I do not suspect an ASF case.
    - ☐ I do not know how to report.
    - ☐ The authorities will check our documents and number of hunted animals.
    - ☐ I always report it.
    - ☐ Other: __________________.

22. What would you think if hunter colleagues report a suspicious case of ASF? Select the closest answer.
    - ☐ Your colleagues are getting themselves into trouble.
    - ☐ Your colleagues are getting into an endless process.
    - ☐ Your colleagues are contributing to detecting a fatal disease for pigs/wild boars in your country.
    - ☐ It is not worth it to report anything to the authorities.
    - ☐ Other:____________.

23. If you hear that a hunter colleague found a dead or sick wild boar in the forest, would you encourage him/her to report the suspicious case to the authorities? Select one answer.
    - ☐ Yes
    - ☐ No

24. Do you think incentives play an important role in reporting? Select one answer.
    - ☐ Yes, without adequate incentive no reporting will happen.
    - ☐ Maybe, an adequate incentive may compensate for the extra hassle that develops after reporting.
    - ☐ No, the hassle of reporting is too big.
    - ☐ No, hunters will report in any case because they believe is their obligation.

25. If financial compensation would be paid for reporting of a carcass or suspected ASF case in wild boar, what would you believe is an appropriate amount? Please, give a number.

26. If you hear that a hunter colleague found a dead or sick wild boar in the forest, would you encourage him/her to report the suspicious case to the authorities? Select one answer.
    - ☐ Yes
    - ☐ No

27. What would be the most convenient way for you to officially report an ASF-suspected case? Select one answer.
    - ☐ Via phone call to a known veterinarian.
    - ☐ Via a phone call to a "hotline" of the veterinary service.
    - ☐ Via internet through a special website of the veterinary service.
    - ☐ Other:______.

28. What is your opinion about the Official State Veterinary Service? Please, indicate your opinion with an "X" for each of the following criteria.

Criteria:	Fully disagree	Disagree	Slightly disagree	Slightly agree	Agree	Fully agree
Competent						
Reliable						
Efficient						
Trustworthy						
Respondent in timely manner						
Easily accessible						

ONE LAST QUESTION

29. How would you grade your honesty in your answers? (5 means perfectly honest)
    ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Thank you very much for your collaboration.

Kind Regards,

The UP-10 Project

Appendix F: KAP Questionnaire for Small Holder Farmers

Knowledge Attitudes and Practices (KAP) Survey- ASF in Ukraine

Questionnaire 3

Target group: Small farms (<1000 pigs).

SECTION 1: DEMOGRAPHIC INFORMATION

1. Give the name of the Oblast, the Rayon and the Village where your farm is located.
   Oblast ______________
   Rayon ______________
   Village ______________

2. How old are you? Select one answer.
   - ☐ ≤20 years old.
   - ☐ 21-35 years old.
   - ☐ 36-50 years old.
   - ☐ 51-65 years old.
   - ☐ ≥66 years old.

3. What is the highest education level of the main person involved in taking care of the pigs? Select one answer.
   - ☐ Primary school
   - ☐ Secondary school
   - ☐ Vocational training
   - ☐ Technical studies
   - ☐ University
   - ☐ Post-graduate

SECTION 2: FARM CHARACTERISTICS

4. About how many pigs are in the farm? Select one option.
   - ☐ ≤100
   - ☐ 101-400
   - ☐ 401-700
   - ☐ 701-1000
5. How would you categorize your farm? As a:
   - ☐ a family-owned backyard farm
   - ☐ a licensed small holder farm
   - ☐ an unlicensed small holder farm
   - ☐ a non-commercial midsized farm
   - ☐ a commercial midsized farm

6. What kind of pigs are in the farm? Select all that apply.
   - ☐ Sows
   - ☐ Boars
   - ☐ Fattening pigs

7. What type of housing do these pigs have? Select one answer.
   - ☐ Outdoor
   - ☐ Indoor
   - ☐ Mixed

8. How are the pigs fed? Select all options that apply.
   - ☐ Pigs are fed with commercial feed for pigs.
   - ☐ Pigs are fed with commercial feed not exclusively for pigs.
   - ☐ Pigs are fed with commercial feed shared with other farms.
   - ☐ Pigs are fed with food leftovers from the farm workers.
   - ☐ Pigs are fed with food leftovers from external sources (e.g. army canteens, catering facilities, prisons, educational institutions).
   - ☐ Other:____________.

9. Do you slaughter pigs at your farm? Select one answer.
   - ☐ Yes (Go to Question 9 and Question 10).
   - ☐ No (Go to Question 11).

10. What are in general the destinations of edible food products produced from pigs slaughtered on your farm? Select all that apply.
    - ☐ I sell it to the local butchery.
    - ☐ I sell it to the market.
    - ☐ I sell it to traders.
    - ☐ The meat is consumed by the employees of the farm.
    - ☐ Other:_____________.

11. What are in general the destinations of non-edible food products produced from pigs slaughtered on the farm? Select all that apply.
    - ☐ Sell them to a third person.
    - ☐ Use them as feed.
    - ☐ Dispose them in a composting pit (pile) together with manure for further use as organic fertilizer.
    - ☐ Dispose them at official dumps.
    - ☐ Dispose them informally (e.g. forest, daily rubbish).
    - ☐ Other:_____________.

12. What is the usual procedure to sell pigs? Select all options that apply.

- ☐ Pigs are sold at a pig/livestock market and a farm-owned vehicle is used for transportation.
- ☐ Pigs are sold at a pig/livestock market and an external transportation company is responsible for transportation.
- ☐ Pigs are sold to private pig owners and a farm-owned vehicle is used for transportation.
- ☐ Pigs are sold to private pig owners and an external vehicle is used for transportation.
- ☐ Pigs are sold to a slaughterhouse directly and a farm-owned vehicle is used for transportation.
- ☐ Pigs are only taken to a slaughterhouse and an external transportation company is responsible for transportation.
- ☐ Other:___________.

13. Select all the biosecurity measures that are used in the farm.

- ☐ The entire area where the pig stables are located is fenced.
- ☐ The entire farm uses an all-in/all-out concept.
- ☐ There is an all-in/all-out concept for each of the different stables.
- ☐ Footbaths with disinfectant are located at the entrance to each pig stable.
- ☐ New pigs are only purchased from known and trusted sources.
- ☐ Farm workers have dedicated work cloths that they leave on the farm.
- ☐ Farm workers have dedicated shoes that they wear for work on the farm that they leave on the farm.
- ☐ Other:____________.

14. How many people in total do normally interact with the pigs on a daily basis?

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ ≥7

15. Do any of the farm workers carry out any of the following activities? Select all that apply.

- ☐ Work in the slaughterhouse.
- ☐ Have pigs at home.
- ☐ Work in a catering facility (e.g. waiter, cook).
- ☐ Work in the forest (e.g. forest-guard).
- ☐ Work in other pig farms.
- ☐ Hunting.
- ☐ Work as a butcher (meat shop).
- ☐ Offer slaughter and butcher services to the private sector.

SECTION 3: KNOWLEDGE OF AFRICAN SWINE FEVER (ASF) IN UKRAINE

16. Select one answer for each of the following questions:

Have you received any training about pig diseases?	☐ Yes	☐ No
Have you ever heard about African Swine Fever (ASF)?	☐ Yes	☐ No
Do you feel you are well-informed about how ASF can be transmitted?	☐ Yes	☐ No
Do you feel confident that you can recognise the clinical signs of ASF?	☐ Yes	☐ No
Do you know anybody who experienced cases of ASF in his/her pigs?	☐ Yes	☐ No

17. Which of the following clinical signs do you associate with ASF in pigs? Select all that apply.
    - ☐ Fever
    - ☐ Diarrhoea
    - ☐ Vesicles around the tongue and lips
    - ☐ Lameness
    - ☐ Reddening or darkening of the skin
    - ☐ High mortality
    - ☐ Vomiting
    - ☐ Lethargy
    - ☐ Difficult to breath
    - ☐ Stillborn or weak piglets
    - ☐ Nervous signs
    - ☐ I do not know any signs

18. Through which of the following pathways can pigs get infected with ASF? Select all that apply.
    - ☐ Through direct contact with a diseased pig or carcass of a diseased pig.
    - ☐ Through direct contact with diseased wild boars or carcass of a diseased wild boar.
    - ☐ Through consumption of kitchen waste.
    - ☐ Through consumption of left-overs from the slaughter process.
    - ☐ Through contact with contaminated manure.
    - ☐ Through contact with contaminated clothing, footwear and/or transport vehicles.
    - ☐ Through a bit of an infected tick.
    - ☐ Through use of contaminated surgical equipment.
    - ☐ Through sexual contact.
    - ☐ Through air-borne transmission.
    - ☐ I do not know any pathways.

19. Which preventive measures are taken on the farm to protect pigs against ASF? Select all that apply.
    - ☐ Quarantine period for new animals in a separate area.
    - ☐ Provision of a salt block.
    - ☐ Vaccination.
    - ☐ Only using commercial pig feed.
    - ☐ Implementing disinfection and cleaning protocols.
    - ☐ Fencing of the farm premise.
    - ☐ Only bring in new pigs from trusted sources.
    - ☐ I do not take any measures.
    - ☐ Other:________.

SECTION 4: SUSPICION OF AN ASF CASE

20. Have you ever suspected ASF on the farm? Select one answer.
    - ☐ Yes
    - ☐ No

21. What can be the reasons that might lead you to **not** suspect ASF in the farm pigs? Select all answers that cover your opinion best.
    - ☐ My oblast is free of ASF.
    - ☐ I'm not sure if I would be able to recognise the clinical signs of ASF in my pigs.
    - ☐ The pigs of the farm are healthy status.
    - ☐ We follow a strict procedure for the destruction of carcasses from diseased pigs.
    - ☐ The pigs of neighbouring farms are healthy so we do not have any reason for concern.
    - ☐ We take care of the food stuff of the pigs.
    - ☐ New pigs come from trustworthy and known places.
    - ☐ There are no wild boars in the farm surroundings.
    - ☐ I always suspect ASF in the pigs.
    - ☐ Other:_________.

22. What would you do if you suspect ASF in the pigs? Select the answers that are closest to your reaction.
    - ☐ Sell it immediately (alive).
    - ☐ Send the animal for immediate slaughter at the slaughterhouse.
    - ☐ Slaughter the animal on the farm immediately and distribute the meat among the workers.
    - ☐ Slaughter the animal on the farm and dispose of the carcase outside of the farm.
    - ☐ Slaughter the animal on the farm and dispose of the carcase within the farm.
    - ☐ Report the suspicion to the veterinarian.
    - ☐ I do not do anything out of the ordinary routine.
    - ☐ Other:_________.

SECTION 5: REPORTING OF AN ASF CASE

23. Have you ever reported a suspected case of ASF? Select one answer.
    - ☐ Yes
    - ☐ No

24. What are the reasons that would **prevent you from reporting** an ASF suspicion? Select all answers that apply.
    - ☐ I do not know how to report.
    - ☐ If I report it, the authorities will kill my pig.
    - ☐ If I report it, it will take ages until I get the financial compensation.
    - ☐ My neighbours would not appreciate it when I report an ASF suspected case.
    - ☐ I do not suspect an ASF case.
    - ☐ I'm not able to recognise a suspected case of ASF in the pigs.
    - ☐ It would affect the sales of my pigs.
    - ☐ I always report it.
    - ☐ Other:__________.

25. How would you feel if you report an ASF-suspicion in your pigs to the authorities that later turned out to be non-infected? Select the closest answer.
    - ☐ Ashamed, my peers will make fun of me. I expected a positive result.
    - ☐ Very happy of not having ASF in the pigs.
    - ☐ Good, I do not care what the test result is, I followed the rules.
    - ☐ Discouraged, I have lost my time and energy.
    - ☐ Relieved, during the waiting period I was very worried.
    - ☐ Other:___________.

26. What would be the most convenient way for you to officially report an ASF-suspicion? Select one answer.
    - ☐ Via phone call to a known veterinarian.
    - ☐ Via a phone call to a "hotline" of the veterinary service.
    - ☐ Via internet through a special website of the veterinary service.
    - ☐ Other:_______.

27. What is your opinion about the Official State Veterinary Service? Please, indicate your opinion with an "X" for each of the following criteria.

Criteria:	Fully disagree	Disagree	Slightly disagree	Slightly agree	Agree	Fully agree
Competent						
Reliable						
Efficient						
Trustworthy						
Respondent in timely manner						
Easily accessible						

ONE LAST QUESTION

28. How would you grade your honesty in your answers? (5 means perfectly honest)

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Thank you very much for your collaboration.

Kind Regards,

The UP-10 Project

Appendix G: Results of the KAP questionnaire to backyard farmers

Table 1. Results of key multiple-choice questions asked of backyard farmers: Household activities, pig feed, and edible food products.

Question	Responses	Answers		
		Selected	Not Selected	Total
(Q5) Are any of these activities carried out by any of the members of your household? (N=227; NA=3)	Work in the slaughterhouse	3 (1.3%)	224 (98.7%)	227
	Hunting	21 (9.3%)	206 (90.7%)	227
	Work in a pig farm	12 (5.3%)	215 (94.7%)	227
	Work in a catering facility (e.g. waiter, cook)	18 (7.9%)	209 (92.1%)	227
	Work in the forest (e.g. forest-guard)	13 (5.7%)	214 (94.3%)	227
	Offer slaughter and butchery services to the private sector	155 (68.3%)	72 (31.7%)	227
	No member of the household carries out any of the aforementioned activities	21 (9.3%)	206 (90.7%)	227
(Q12) Which of these statements are the closest to the feeding habits of your pigs? (N=223; NA=0)	I use commercial feed for pigs	61 (26.2%)	172(73.8%)	233
	I use commercial animal feed but not exclusively for pigs	26 (11.2%)	207 (88.8%)	233
	I use kitchen leftovers	125 (53.6%)	108 (46.4%)	233
	I produce my own pig feed	165 (70.8%)	68 (29.2%)	233
(Q12) Which of these statements are the closest to the feeding habits of your pigs? (N=223; NA=0)	I share the commercial feed for pigs with my neighbours	3 (1.3%)	230 (98.7%)	233
	My neighbour and I share the kitchen leftovers to feed our pigs	4 (1.7%)	229 (98.3%)	233
	Other	5 (2.1%)	228 (97.9%)	233
(Q14) What do you normally do with the edible food products after slaughtering the pigs? (N=231; NA=1)	Consumption within my household	200 (86.6%)	31 (13.4%)	231
	Give it to neighbours/relatives	72 (31.2%)	159 (68.8%)	231
	Sell it to the local butchery	11 (4.8%)	220 (95.2%)	231
	Sell it to the market in my village or in a neighbouring village	43 (18.6%)	188 (81.4%)	231
	Sell it to traders	37 (16%)	194 (84%)	231

Question	Responses	Answers		
		Selected	Not Selected	Total
	Others	0 (0%)	231 (100%)	231

Table 2. Results of key multiple-choice questions asked of backyard farmers: Non-edible food products and ASF reporting.

Question	Responses	Answers		
		Selected	Not Selected	Total
(Q15) What do you normally do with the non-edible food products after slaughtering the pigs? (N=224; NA=9) ***(Q15) What do you normally do with the non-edible food products after slaughtering the pigs? (N=224; NA=9)***	Sell them to a third party	3 (1.3%)	221(98.7%)	224
	Use them as feed	76 (33.9%)	148 (66.1%)	224
	Dispose of them in a composting pit (pile) together with manure for further use as an organic fertilizer)	120 (53.6%)	104 (46.4%)	224
	Dispose of them at official dump	21 (9.4%)	203 (90.6%)	224
	Dispose of them informally (e.g. forest, daily rubbish bin)	27 (12.1%)	197 (87.9%)	224
	Other	18 (8%)	206 (92%)	224
(Q27) What are the reasons that would prevent you from reporting an ASF suspicion? (N=227; NA=2)	I do not know how to report	43 (18.9%)	184 (81.1%)	227
	If I report it, the authorities will kill my pig	28 (12.3%)	199 (87.7%)	227
	If I report it, it will take ages until I get the financial compensation	29 (12.8%)	198 (87.2%)	227
	I'm not able to recognize a suspected case of ASF in the pigs	52 (22.9%)	175 (77.1%)	227
	It would negatively affect the sales of my pigs	26 (11.5%)	201 (88.5%)	227
	My neighbours would not appreciate it when I report an ASF suspicion	20 (8.8%)	207 (91.2%)	227
	I always report it	63 (27.8%)	164 (72.2%)	227
	I have never suspected an ASF case	93 (41%)	134 (59%)	227
	Other	4(1.8%)	223(98.2%)	227

Table 13. Results of key single-choice questions asked of backyard farmers.

Question	Response	Number	Frequency (%)
(Q12) How are your pigs slaughtered? (N=233; NA=0)	Home slaughter without veterinary inspection	179	79.20
	Home slaughter with veterinary inspection	30	13.72
	At the slaughterhouse	16	7.08

Table 3. Results of key single-choice questions asked of backyard farmers.

Question	Responses	Number	Frequency (%)
(Q16) Do you feel you are well-informed about how ASF can be transmitted? (N=221; NA=12)	Yes	104	47.06%
	No	117	52.94%
(Q16) Do you feel confident that you can recognize the clinical signs of ASF? (N=220; NA=13)	Yes	70	31.82%
	No	150	68.18%
(Q26) Have you ever reported a suspected case of ASF on your premises? (N=228; NA=5)	Yes	15	6.58%
	No	213	93.42%

Appendix H: Results of the KAP survey for wild boar hunters

Table 1. Results of key multiple-choice questions asked of wild boar hunters.

Question	Responses	Answers		
		Selected	Not selected	Total
(Q9) What do you normally do with the wild boars that you have hunted? (N=184; NA=1)	I use the meat for home consumption	156 (84.8%)	28 (15.2%)	184
	I give the meat to neighbours/relatives/friends	57 (31%)	127 (69%)	184
	I sell it on the local market	0 (0%)	184 (100%)	184
	I sell it to a trader	1 (0.5%)	183 (99.5%)	184
	I do not use it at all	1 (0.5%)	183 (99.5%)	184
	I leave it at the shooting site (forest) as feed for predatory animals	1 (0.5%)	183 (99.5%)	184
	I leave it in a closed waste pit in the forest	32 (17.4%)	152 (82.6%)	184
	I bring the whole carcass to an equipped point for dressing and further processing	17 (9.2%)	167 (90.8%)	184
	This is not my business; I do not think about it	5 (2.7%)	179 (97.3%)	184
(Q21) What are the reasons that would prevent you from reporting an ASF suspicion? (N=183; NA=3)	My hunting colleagues would not appreciate it	6 (3.3%)	177 (96.7%)	183
	I do not want to waste hunting time by dealing with the authorities	18 (9.8%)	165 (90.2%)	183
	The authorities will take the wild boar carcass	3 (1.6%)	180 (98.4%)	183
	If I report it, it will take ages until I get the financial compensation	42 (23%)	141 (77%)	183
(Q21) What are the reasons that would prevent you from reporting an ASF suspicion? (N=183; NA=3)	I do not suspect and ASF case	58 (31.7%)	125 (68.3%)	183
	I do not know how to report	23 (12.6%)	160 (87.4%)	183
	The authorities will check our documents and number of hunted animals	12 (6.6%)	171 (93.4%)	183
	I always report it	50 (27.3%)	133 (72.7%)	183
	Other	8 (4.4%)	175 (95.6%)	183

Table 2. Results of key single-choice questions to wild boar hunters.

Question	Responses	Number	Frequency (%)
(Q8) When you go hunting and bring your own food supplies for the day, what would you do with leftover food? (N=187; NA=1)	I typically throw it away in the environment	52	27.81
	I carry it back home	76	40.64
	I typically throw it away at an official disposal location	49	26.20
	Other	10	5.35
(Q22) What would you think if a hunter colleagues report a suspicious case of ASF? (N=182; NA=6)	Your colleagues are getting themselves into trouble	11	6.04
	Your colleagues are getting into an endless process	32	17.58
	Your colleagues are contributing to detecting a fatal disease for pigs/wild boars in your country	136	74.73
	It is not worth it to report anything to the authorities	0	0
	Other	3	1.65
(Q24) Do you think incentives play an important role in reporting? (N= 179; NA=9) ***(Q24) Do you think incentives play an important role in reporting? (N= 179; NA=9)***	Yes, without adequate incentive no reporting will happen	82	45.81
	Maybe, an adequate incentive may compensate for the extra hassle that develops after reporting	52	29.05
	No, the hassle of reporting is too big	3	1.68
	No, hunters will report in any case because they believe it's their obligation	42	23.46

Table 3. Results of key single-choice questions to wild boar hunters.

Question	Responses	Number	Frequency (%)
(Q11) Do you feel you are well-informed about how ASF can be transmitted? (N=185; NA=3)	Yes	46	24.86
	No	139	75.14
(Q11) Do you feel confident that you can recognize the clinical signs of ASF? (N=185; NA=3)	Yes	27	14.59
	No	158	85.41
(Q20) Have you ever reported a suspected case of ASF? (N=185; NA=3)	Yes	5	2.7
	No	180	97.30
(Q23) If you hear that a hunter colleague found a dead or sick wild boar in the forest, would you encourage him/her to report the suspicious case to the authorities? (N= 181; NA= 6)	Yes	153	84.53
	No	28	15.47

Appendix I: Publications and Presentations

(1) UP-10 Presentations at the DTRA Science Program Review, 19-20 September 2019, Poland

- **UP-10 Abstract for oral presentation**

UP-10 "Regional Field-to-Table Risk Assessment of the spread of African swine fever virus (ASFV) across Ukraine in wild fauna and via consumer trade routes – insight into the development of effective ASFV quarantine strategies and public policy"

Mykola Sonko[1], Mykola Sushko[2], Hanna Kovalenko[3], Usachenko Nataliia[2], Oleksii Solodiankin[4], Rudova Nataliia[4], Larysa Muzykina[3], Buzun Andrii[4], Maksym Bezimennyi[3], Skorokhod Serhii[2], Maryna Sapachova[2], ZInaida Klestova[5], Oleksii Kudriavchenko[5], Volodymyr Polishchuk[6], Andriy Shelepylo[7], Mykola Sytiuk[3], Serhii Nychyk[3], Anton Gerilovych[4], Andrii Mezhenskyi[2], Marco De Nardi[8], Manon Schuppers[8], Karen Saylors[9], Stephen Higgs[10]

[1]State Service of Ukraine for Food Safety and Consumer Protection;
[2]State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Ukraine;
[3]Institute of Veterinary Medicine of the NAAS of Ukraine;
[4]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Ukraine;
[5]State Scientific Control Institute of Biotechnologies and Strains of Microorganisms, Ukraine;
[6]National University of Life and Environmental Sciences, Ukraine;
[7]State Forest Resources Agency of Ukraine;
[8]SAFOSO AG, Switzerland;
[9]Labyrinth Global Health, Inc., USA;
[10]Kansas State University, USA.

ASFV is a highly infectious agent that causes a devastating and frequently fatal disease African swine fever (ASF) of swine. The disease outbreaks often inflict significant economic loss due to the widespread culling of affected animals, production losses, and implementation of trade restrictions to prevent further viral spread within the region and across regional borders. As a potential transboundary disease capable of severe economic damage, ASFV is a significant concern within the European Union (EU) and neighboring countries, including Ukraine. In Ukraine and neighboring countries, anthropogenic factors and poor biosecurity measures most likely represent the biggest contributors to the rapid spread of ASFV. Supporting this hypothesis is the observation that transmission has been associated with major transportation and trade corridors from the North to the South and the East to the West within the territory of Ukraine.

UP-10 project seeks to expand upon research and biosurveillance efforts currently underway within Ukraine in order to inform effective biosecurity strategies and the development of public policy for controlling the spread of ASFV within Ukraine. Through connecting biosurveillance findings and policy development, the overall outcome of this program will be recommendations for the development of policy and public outreach designed to limit the potential for ASFV transmission via consumer trade routes and/or across regional borders

through human activities. Furthermore, due to the imperative to quickly resolve the growing ASF crisis in Ukraine, this project will link Ukrainian officials with regional researchers and ASFV control programs in order to build upon best practices and lessons learned for blocking the spread of ASF further into European Union countries. Implementing the findings of this project will contribute to the economic sustainability and stability of agricultural markets within Ukraine and contribute to Ukraine's further integration and alignment with EU trade and policy. As such, the project consists of three focus "Project Pillar" areas that will be undertaken in parallel: A) Defining Geographical and Zoonotic Factors, B) Tracking Anthropogenic and Socio-Economic Factors, and C) Public Policy/Communications.

The main purpose of the project is to assess the relationship between anthropogenic, socio-economic and environmental risk factors, their contribution to and impact on ASFV distribution/spread in Ukraine and develop public policy/communications that will reduce the rate of ASFV spread to new areas and across international borders.

During first two quarters of the project implementation, the following tasks were addressed:

- Ukrainian researchers and SMEs from SAFOSO, Metabiota, Labyrinth, and KSU coordinated points-of-contact, communication, and scientific working groups to pursue project objectives.
- Outreach Working Group Meeting was implemented.
- Policy Working Group Meeting was implemented.
- Implementation plan for the Knowledge Attitudes and Practices (KAP) survey was designed.
- Three KAP questionnaires for backyard farmers, small holder farmers, and wild boar hunters were developed.
- SSUFSCP, in coordination with NULES, initiated development of the UP-10 Project website as a collective platform for project participants. Additionally, selected sections of this website will be available for public access and will serve as the ASF public awareness tool.
- SOPs for field sample collection, sample transportation, storage, and testing by qPCR were developed by SMEs and Ukrainian participating institutions.

Implementation of the project will contribute to threat reduction on the following directions:

- Increasing regional understanding of the risk posed by ASFV in Ukraine and for communicating and applying those risk models to neighbors and international partners.
- Reducing risk of transboundary spread of ASFV and of the subsequent threat to economic stability, either into or from Ukraine to the European Union (EU) and other international trade partners.
- Improving ability of the regional biosurveillance network to respond to, and prevent the spread of, future transboundary disease threats.

(2) UP-10 Abstract submitted for 2020 ROHRS

- Abstract

The Cooperative Biological Research Project UP-10 as the Next Stage in Measures against ASF for Ukraine

Polishchuk V.[1], Sonko M.[2], Solodiankin O.[3], Rudova N.[3], Gerilovych A.[3], Nychyk S.[4], Hudz N.[4], Pavlenko A.[5], Mustra D.[5], Saylors K.[6], Muñoz V.[7], De Nardi M.[7], Schuppers M.[7]

[1]*National University of Life and Environmental Sciences of Ukraine;*
[2]*SSUFSCP;*
[3]*NSC Institute of Experimental and Clinical Veterinary Medicine of the NAAS of Ukraine;*
[4]*Institute of Veterinary Medicine of the NAAS of Ukraine;*
[5]*Metabiota Inc., USA;*
[6]*Labyrinth Global Health, USA;*
[7]*SAFOSO AG, Switzerland*

The analysis of the causes and factors affecting the long-term disadvantage of Ukraine with regard to ASF requires assessment of the population's awareness on the threat of the disease and the study of the ways of its probable introduction, spread and distribution throughout the country. It is well known that pop-up markets can play a significant role in this process. However, the absence of objective statistics, both in terms of the number of such sites in Ukraine and the nature and volume of products sold there, makes such analysis impossible. Given that publicity and transparency is the key to successful implementation of the UP-10 project, the team initiated the creation of a dedicated specialized web resource www.up10.vet.ua, based on best practice algorithms for promoting the knowledge on ASF risk.

In order to develop an effective national outreach strategy, the study on knowledge, attitude, and practice (KAP survey) was conducted. The research resulted in identifying weaknesses in the ASF countermeasure system in the country and generating directions that need to be explored for an increased impact strategy. An objective, impartial assessment of the level of awareness among the various categories of pig breeding citizens has identified the least informed categories of the population and has given rise to greater impact through media as well as targeted web resources and social networks, as well as enhanced training for professionals and students, involving leading scientists. Coordination of the project participants in the sampling of pork at pop-up markets, with their further testing for ASF, will allow an objective assessment of the impact of trade routes on the spread of the disease and will serve as a basis for appropriate adjustments. Data entry is recorded in mobile applications created on the KoBoToolbox. New technologies of KAP survey for data collection, analysis, sampling, and knowledge dissemination, using GIS components and mobile applications allow professionals to significantly reduce time and focus on production needs, and provide citizens with yet another additional source of reliable information on measures against ASF.

(3) UP-10 Abstracts submitted for GARA

- **Abstract 1**

Investigating the Anthropogenic Contribution to the Spread of African Swine Fever virus (ASFV) in Ukraine through the Illegal Backyard and Non- Commercial Trade of Meat Products.

Andrii Mezhenskyi[1], Volodymyr Polishchuk[2], Serhii Nychyk[3], Anton Gerilovych[4], Andrii Pavlenko[5], David Mustra[5], Karen Saylors[6], Stephen Higgs[7], Mykola Sonko[8]

State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine[1]; National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine[2]; Institute of Veterinary Medicine, Kyiv, Ukraine[3]; National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine[4]; Metabiota, San Francisco, CA, USA[5]; Labyrinth Global Health, Saint Petersburg, FL, USA[6]; Biosecurity Research Institute, Kansas State University, Manhattan, KS, USA[7]; State Service of Ukraine on Food Safety and Consumer Protection, Kyiv, Ukraine[8];

In Ukraine, anthropogenic factors and poor biosecurity measures represent the biggest contributors to the rapid spread of African Swine Fever virus (ASFV). Transmission has been associated with major transportation and trade corridors from North to South and East to West within Ukraine. Anthropogenic and socio-economic factors that may contribute to the spread of ASFV were investigated through biosurveillance activities, including surveys and the collection of domestic pork products procured from illegal rural vendors and unlicensed sales points. Samples targeted for molecular diagnostics testing included both unprocessed pig meat and organs (spleen, lymph nodes, liver, tonsil, heart, lung, and kidney) and processed pig meat (sausage). Sampling was conducted in regions that either demonstrated unique overlap of wild boar and domestic swine outbreaks, or have an increased risk of contributing to transboundary spread of ASFV due to their proximity to international borders. These regions include Zakarpattia, Rivne, Kharkiv, and Odesa Oblasts. Roughly 2000 meat and organ samples were collected by State representative field teams. All samples were handled according to Ukrainian requirements for the handling of materials potentially contaminated with ASFV. DNA for ASFV analysis was extracted using commercial PCR kits produced by Sivital (Republic of Belarus). At the time of purchase, field teams collected informal survey data from each vendor along with geolocation data. Collected data was uploaded to the project website for tracking and analysis of GIS mapping to establish ASF disease distribution and develop forecasts. The project demonstrated anthropogenic factors contributing to the spread of ASF for the first time in Ukraine and provided insight into existing gaps in the biosurveillance system that should be addressed in order to implement effective biosecurity and control measures for preventing farm-to-farm and farm-to-wildlife spread.

- **Abstract 2**

UP10 – Building scientific evidence for improved ASF surveillance and control in Ukraine

Volodymyr Polishchuk[1], Mykola Sonko[2], Oleksii Solodiankin[3], Nataliia Rudova[3], Anton Gerilovych[3], Serhii Nychyk[4], Nataliia Hudz[4], Andrii Pavlenko[5], David Mustra[5], Karen Saylors[6], Violeta Muñoz[7], Marco De Nardi[7], Manon Schuppers[7]

National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine[1]; State Service of Ukraine on Food Safety and Consumer Protection, Kyiv, Ukraine[2]; National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine[3]; Institute of Veterinary Medicine, Kyiv, Ukraine[4]; Metabiota, San Francisco, CA, USA[5]; Labyrinth Global Health, Saint Petersburg, FL, USA[6]; SAFOSO AG, Switzerland[7]

A comprehensive and effective national ASF control strategy for Ukraine requires a good understanding of the underlying factors for ASF. Epidemiological data about reported outbreaks are available, but there are knowledge gaps concerning risk behavior of backyard pig owners and hunters and concerning the role of illegal domestic pig meat trade in the spread of ASF. UP-10 aims to generate data about these important driving factors for continued ASF spread and make these data available to policy makers for decision-making.

A knowledge, attitude and practice (KAP) survey was conducted among backyard pig owners and hunters in several oblasts to gain more insights in their knowledge of and response towards ASF. The results showed that in particular backyard pig owners displayed many risk behaviors that would contribute to sustained ASF spread if the infection were present. A survey of pig meat obtained from illegal sales points provided more insights in the abundance of contaminated pig meat on the domestic market, which could facilitate its way back into domestic pigs or wild boar through risk behavior of people. The survey made use of a newly developed mobile application (KoBoToolbox) to record the geographical sampling location and additional information about the sample. Further, the project strengthened the capacity of risk managers to use spatial analytical methods to investigate the pattern of ASF in the country, generating relevant epidemiological information for decision-making.

Transparent communication of results is fundamental to enable policy makers and the general public to better understand the impact of ASF and the importance of comprehensive disease control. For this purpose, UP-10 developed a dedicated website (www.up10.vet.ua) through which ASF information can be disseminated. This online communication tool can be complemented with additional information and outreach activities, including training, media releases or printed publications which were prioritized by stakeholders during ad-hoc workshops.

Appendix J: Outreach Working Group Meeting

Topic: Report about the Outreach Working Group Meeting, 12-13 June 2019, Kyiv, Ukraine

I. Executive Summary

An Outreach Working group meeting took place in Kyiv at Riviera Hotel, conference hall "Chapter", on 12-13 June 2019, Kyiv, Ukraine. The man objective of this meeting is to continue to work on the development of an effective national public outreach strategy with a focus on survey design and implementation for improving understanding of the influence of anthropogenic factors on the spread of ASFV. During the 2-day event participants discussed past and on-going outreach activities in the country, presented completed and on-going projects, discussed the current epidemiological situation related to ASF in Ukraine and other countries, revised educational materials that need to be developed and implementation strategy of the outreach programs for the target groups. During this meeting, Volodymyr Polishchuk from NULES presented a UP-10 project related website that he developed. Additionally, Dr. Mustra met with the representative of SSUFSCP (Mykola Sonko) to discuss UP-10 project implementation activity in Ukraine. Information on the project participants, agenda and main result of working groups are presented below.

II. Specific objectives:

1) To revise the current epidemiological situation related to ASF in Ukraine and other regions
2) To identify target groups (e.g. farmers, hunters) to be reached by the outreach programs
3) To define the implementation strategy of the outreach programs for each target group
4) To identify what educational materials needs to be revised/developed in UP-10

III. Panel

Name	Institution
Marco De Nardi	SAFOSO AG
Violeta Munoz	SAFOSO AG
David Mustra	BVSPS/Metabiota

IV. Participants

#	Name	Institution
1	Zinaida Klestova	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms
2	Oleksii Kudriavchenko	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms

#	Name	Institution
3	Andrii Mezhenskyi	The State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)
4	Maksym Bezymennyi	Institute of Veterinary Medicine
5	Serhii Nychyk	Institute of Veterinary Medicine
6	Mykola Sytiuk	Institute of Veterinary Medicine
7	Yuliia Glukhonets	State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP)
8	Mykola Sonko	State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP)
9	Vitalii Nedosekov	National University of Life and Environmental Sciences (NULES)
10	Volodymyr Polishchuk	National University of Life and Environmental Sciences (NULES)
11	Iryna Makovska	National University of Life and Environmental Sciences (NULES)
12	Valeriia Yusteniuk	National University of Life and Environmental Sciences (NULES)
13	Nataliia Rudova	National Scientific Center “Institute of the Experimental and Clinical Veterinary Medicine” (IECVM)
14	Yurii Dunaiev	National Scientific Center “Institute of the Experimental and Clinical Veterinary Medicine” (IECVM)

V. UP-10 Web-site (http://www.up10.vet.ua/)

Dr. Volodymyr Polishchuk from NULES initiated creation of public resources (web-site) related to UP-10 project. This is a platform to inform public about collaborative activity of project participants. This platform is also could be an instrument to control locations of swine products and KAP survey efforts with the use of mobile applications. Currently, the Web-site is under construction and includes the following main sections for testing:

1. General information about the project
2. Resources (ASF related materials - publications, presentations, photos, etc.)
3. Map (Ukrainian map with indication of target territories for study)
4. Registration sheet (proposed structure of data to be collected and recorded)
5. Questionnaire (proposed structure of data to be recorded during KAP survey)
6. Sample collection using mobile application
7. Questionnaire for hunters and possibility for data entry with the use of mobile application
8. Questionnaire for farmers and possibility for data entry with the use of mobile application

Additionally, Dr. Polishchuk raised the following questions for consideration and confirmation:

1. Logo (what logos could be indicated, approval for their indication, etc.)
2. Summary of project description on the main page (confirmation of the content).
3. Detailed project description (a separate page) – description of project participants, statement of work (SOW), timeline, etc.
4. Up to 9 slides about the project to be uploaded on the main page (additional resources are required to include more than 10 slides on the main page).
5. List of participating organizations involved in the project.
6. List of Project Participants/Principal Investigators (photos, title, role in the project, etc.) for public access.
7. Information about the leaders of the groups.
8. Subject Matter Experts (similar to asfld.vet.ua).
9. Timeline of project's events (meetings, round table discussions, workshops, training, etc.).
10. The structure of administrative resources and level of access (POC responsible from SSUFSCP and State Forest Resources Agency of Ukraine (SFRA)).

Stages for the development of public resources related to UP-10 project

1. Registration of subdomain (www.up10.vet.ua).
2. Establishment of CMS.
3. Establishment of additional modules, components and their adjustment.
4. Preparation of template/structure for data entry into the system.

Creation of sample/data documentation tools.

1. Identification and approval of sampling areas/locations.
2. Approval of the strategy/format and list of individuals for data entry into the system using mobile applications
3. Approval of the laboratories for storage of samples to be collected and SSRILDVSE as a reference laboratory in the country for laboratory study.
4. List of individuals that are authorized to collect sample and entry data into the system for further public awareness.
5. Approval of orders from corresponding authorities - State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP), State Forest Resources Agency of Ukraine (SFRA) for project implementation in Ukraine (main departments, regional laboratories, research institutions, etc.).
6. Standard Operation Procedures (SOP) for sample collection, packing, labeling, transportation, storage, laboratory analysis, data analysis, etc.
7. Testing of system prototype, training for members of the groups to be involved in sampling activity and KAP survey efforts (communication plan, photo records and data entry into the system).

Appendix K: Online session on GIS and Spatial analysis

Virtual Conference through Microsoft Teams platform, 01 July 2020, Kyiv, Ukraine

I. Participants

#	Name	Institution
1	Oleksandr Napnenko	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM)
2	Nataliia Mezhenska	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM)
3	Roman Datsenko	State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)
4	Maksym Bezymennyi	Institute of Veterinary Medicine (IVM)
5	Vitalii Nedosekov	National University of Life and Environmental Sciences (NULES)
6	Volodymyr Polishchuk	National University of Life and Environmental Sciences (NULES)
7	Iryna Makovska	National University of Life and Environmental Sciences (NULES)
8	Oksana Zlenko	National Scientific Center “Institute of the Experimental and Clinical Veterinary Medicine” (IECVM)
9	Serhii Filatov	National Scientific Center “Institute of the Experimental and Clinical Veterinary Medicine” (IECVM)

Appendix L: UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences

Microsoft Teams Platform, 19-25 June 2020, Kyiv, Ukraine

Topic: Report about the UP-9 OY1/UP-10 CBR Project Close-Out Meetings Virtual Conferences, Microsoft Teams Platform, 19-25 June 2020, Kyiv, Ukraine

I. Executive Summary

Taking into account the Ukrainian national restriction policy and quarantine measures due to COVID-19, UP-9 OY1/UP-10 CBR Project Close-Out Meetings, were held online through Microsoft Teams platform, 19-25 June 2020, Kyiv, Ukraine.

The main objective of the online meetings was to review and discuss findings and accomplishments regarding ASF surveillance, including efforts focused on, but not limited to, capacity development, surveillance actions, forecasting, amendments to regulations for regional data sharing in support of an ASF collaborative network, and strategies for continued regional collaborative activities.

During the online sessions, participants discussed success and challenges, identified areas for improvement, lessons learned, as well discussed follow-up activities and recommendations for future projects.

Attendees of the Close-out meetings reached the conclusion that specialists in Ukraine have received a wonderful tool in the form Knowledge, Attitudes and Practice (KAP)-research methods and are able to use this tool independently and train future specialists. Additionally, Volodymyr Polishchuk from NULES underline that the methods of KAP-research are included in the Master's program in the course: “Information technologies in the veterinary medicine.

Information on the project participants and agenda are presented below.

II. Specific objectives:

1. To present the outcomes of and accomplishments of the UP-10 implemented in Ukraine.
2. To discuss challenges encountered during the implementation of the project.
3. To discuss the implementation and sustainability of future research activities as part of a wider national ASF prevention strategy.

III. Participants

#	Name	Institution
1	Zinaida Klestova	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM)
2	Oleksii Kudriavchenko	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM)
3	Nataliia Mezhenska	State Scientific Control Institute of Biotechnologies and Strains of Microorganisms (SSCIBSM)
4	Andrii Mezhenskyi	State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)
5	Serhii Skorokhod	State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)
6	Anna Kyivska	State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)
7	Maksym Bezymennyi	Institute of Veterinary Medicine
8	Oleksandr Trarasov	Institute of Veterinary Medicine
9	Serhii Nychyk	Institute of Veterinary Medicine
10	Nataliia Hudz	Institute of Veterinary Medicine
11	Mykola Sonko	State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP)
12	Oleksandr Arnaut	SSUFSCP Odesa Oblast
13	Andrii Rusin	SSUFSCP Zakarpattia Oblast
14	Yaroslav Rebets	SSUFSCP Zakarpattia Oblast
15	Volodymyr Novosad	SSUFSCP Rivne Oblast
16	Oleksandr Kostyuk	SSUFSCP Rivne Oblast
17	Iryna Khrystoyeva	SSUFSCP Kharkiv Oblast
18	Evhen Tinyaev	SSUFSCP Kharkiv Oblast
19	Volodymyr Polishchuk	National University of Life and Environmental Sciences (NULES)
20	Valeriia Yustynyuk	National University of Life and Environmental Sciences (NULES)
21	Anton Gerilovych	National Scientific Center "Institute of the Experimental and Clinical Veterinary Medicine" (IECVM)
22	Oleksii Solodiankin	National Scientific Center "Institute of the Experimental and Clinical Veterinary Medicine" (IECVM)
23	Nataliia Rudova	National Scientific Center "Institute of the Experimental and Clinical Veterinary Medicine" (IECVM)
24	Andrii Buzun	National Scientific Center "Institute of the Experimental and Clinical Veterinary Medicine" (IECVM)

IV. Agenda

Day 1, Virtual Conference 1, Friday, 19 June 2020		**Meeting leads**
14.00-16.30 EET (Kyiv time)	• Welcome, introduction to the meeting, and general objectives • Overview of manuscripts initiated within UP-10 project and getting consensus on next steps leading to their completion: (1) Supporting control policies on African swine fever in Ukraine through a knowledge, attitudes and practice (KAP) survey targeting backyard farmers (2) [To be titled] Review of market purchases and seller practices from informal surveys (3) [To be titled] Reporting on the fining of ASFV positive samples in products purchase at illegal markets (4) [To be titled] In depth analysis on the findings of the UP-10 project including GIS data and review of biosafety measures encountered at sales sites • Wrap-up and concluding remarks	SAFOSO/NULES/SSRILD VSE/IECVM/IVM/ UP-10 team
Day 2, Virtual Conference 2, Tuesday, 23 June 2020		
14.00-16.30 EET (Kyiv time)	• Welcome, introduction to the meeting, and general objectives • Overview of findings on anthropogenic factors contributing to the spread of ASF in Ukraine from market purchases and seller practices from informal survey and KAP survey • Development of outreach campaigns and identification and addressing the gaps of the current outreach strategy • Next steps of implementing public outreach strategy in Ukraine	KSU/Labyrinth/SAFOSO/NAAS/UP-10 team
Day 3, Virtual Conference 3, Thursday, 25 June 2020		
09.00-11.30 EET (Kyiv time)	• Welcome, introduction to the meeting, and general objectives • Identification of critical policy factors • International approaches for ASF control strategy • ASF control strategy implementation in Ukraine (based on project findings and international examples) • Creation of a publicly accessible UP-10 web site with sub-site for members of the Public Policy and Communications working groups	Labyrinth/ KSU/SAFOSO/All/ UP-10 team
Day 4, Virtual Conference 8, Tuesday 30, June 2020		
09.00-11.30 EET (Kyiv time)	• Welcome, introduction to the meeting, and general objectives • Future ASF and informal trade-network project proposal development • Wrap-up and concluding remarks	All

Appendix M: Kit protocol for Belarusian-produced test system from Sivital

Please refer to the pdf version of the Final Report for the manufacturer’s original Russian language protocol and an English translation (unofficial) of the original Russian language protocol for the following product:

ТУ BY 391360704.011-2015 (100 tests) /(50 tests)

LLC "Sivital"
210017, Republic of Belarus, Vitebsk,
st. Gagarin, 11, building 12
Tel .: + 375-212-23-20-07
Fax: + 375-212-23-14-48
E-mail: info@sivital.by

TEST SYSTEM FOR DETECTING ASFV DNA VIRUS BY REAL-TIME PCR METHOD
TU BY 391360704.011-2015
INSTRUCTIONS FOR APPLICATION.

Designed for 100/50 quality ASFV DNA determinations
(all delivery options are listed)

Universal format

Designed for use with
block and carousel
thermal cyclers capable of working with
samples with a volume of 25 µl and
register FAM / HEX fluorescence,
e.g. CFX96 TM , Rotor-Gene

TU BY
391360704.011-2015 (100
tests) /
(50 tests)

Recommended storage temperature

LLC "Sivital"
210017, Republic of Belarus, Vitebsk,
st. Gagarin, 11, building 12

Tel .: + 375-212-23-20-07
Fax: + 375-212-23-14-48
E-mail: info@sivital.by

System test content

Table 1: Components of the test system

Marking	Packaging (50 tests)	Packaging (one hundred tests)	View packaging	Description
Kit for NK allocation	50	one hundred	Box / bag with components recruitment	Nucleic Extraction Kit acids from biological material
ASFV-Oligo-nucleotide mixed	2 bottles	3 bottles	amber bottle, amber cap	Lyophilized mixture of reagents, containing specific primers ASFV , DNA probes, mix deoxyribonucleotides, and oligonucleotides
ASFV standard	2 standard	4 standard	-	Concentration standards tubes, containing synthetic ASFV DNA
Water for PCR-research (EYE)	2 × 1.5 ml	4 × 1.5 ml	colorless bottle, colorless cap	Purified water for PCR
50 × ROX	1 × 0.20 ml	1 × 0.20 ml	amber bottle, violet cap	50 × passive concentrate reference dye ROX
Taq-polymerase	1 × 135 Unit	2 × 135 units	colorless bottle, colorless cap	Enzyme Taq-polymerase (5 U / µl)
Buffer mix2	1 × 0.5 ml	2 × 0.5 ml	colorless bottle, orange cap	10 × PCR buffer concentrate, containing magnesium chloride
IN TO	2 × 0.3 ml	4 × 0.3 ml	-	Internal standard tubes, containing synthetic DNA
	1	1	Instructions	

Storage conditions, stability

The ASFV detection kit can be shipped at room temperature for
excluding Taq polymerase and 10x PCR buffer concentrate, which should
transported on dry ice. Store the entire kit (including Taq polymerase and 10 × PCR
buffer) must be in a dark place at -20 ... -30 ° C, where it should be placed immediately after
delivery. The stability of the kit is guaranteed during the entire shelf life (with
storage under the conditions specified above). Reagents not included in the kit should
stored under the conditions recommended by their manufacturers.

Remark . Required amount of reagent mixture (ASFV-oligonucleotide mixed)
immediately before use, it must be dissolved in water for PCR-
research. The remainder of the dissolved reagent mixture can be stored at + 2 + 8 ° C in
for at least 3 months (do not freeze! always protect from light!)

Restrictions on product use

A very high concentration of heparin in the sample can lead to critical
decrease in the coefficient of extraction of copies of ASFV DNA. When using samples different
nature may produce incorrect results. The test system can be
used only with specified PCR equipment.

Collection and storage of samples

Collection and processing of clinical samples

Blood (plasma, serum)

Collect 5-10 ml of peripheral blood using standard tubes for
collecting samples.
Anticoagulant coated blood collection tubes must be used
EDTA or citrate.
It is allowed to take blood into standard tubes with pre-added
3% solutions of the above coagulants at a rate of 10: 1.
Centrifuge the blood at 1500 rpm for 10 min (obtaining plasma).
To obtain blood serum, blood is taken without an anticoagulant

Thick fabrics

Homogenize 25 mg tissue in the presence of 250 µl saline or phosphate
buffer.
The isolated DNA can be stored in deep freeze conditions (-20 ... -80 ° C) in
for several months; storage is allowed at + 2 ... + 8 during the day.

Chemical interactions

Elevated levels of bilirubin, lipids, hemoglobin, EDTA (≤30 mM), and citrate

(≤3.13%) do not affect the performance of the test system.

Heparin (40 mg / dL) inhibits PCR.

User-supplied reagents and equipment

Thermocycler for Real-Time PCR.
Thermal cycler software for data analysis and logging.

Plastic consumables for thermal cycler (RNA-se / DNA-se Free).
Pipettes, sterile tips with aerosol barrier (RNA-se / DNA-se
Free).
High speed microcentrifuge suitable for 0.2 ml, 1.5 ml and 2.0 ml tubes
ml as well as for 96-well plates.
Solid state oven suitable for 0.2 ml, 1.5 ml and 2.0 ml tubes,
as well as for 96-well plates.
Vortex.
1.5 ml tubes.
Gloves, lab coat.

Isolation of DNA from a sample of serum and blood plasma (column method).

Carried out using the kit for the isolation of nucleic acids (NK) included in composition of the test system.

Table 2.1: Kit components.

Name	amount for 50 samples	Description
Lysis buffer	35 ml	
Wash buffer 1	30 ml	
Wash buffer 2 (concentrate)	12 ml	
Water for PCR studies	13 ml	pH about 7 - 8
Eluent	13 ml	pH 8.5
RNA transporter	1 mg	Lyophilized
Proteinase buffer	1.8 ml	
Proteinase K	30 mg	Lyophilized
Column filter	50	Dark blue rings
NDT sampling tubes	4x50	Collection tubes (2 ml) included
Sample tubes	50	Sample tubes (2.0 ml) containing stabilized internal controls included with the test system
Elution tubes	50	Elution tubes (1.5 ml)

Required reagents and equipment

96-100% ethanol (to improve nucleic acid precipitation and to preparation of wash buffer 2). Only brands "rubbing alcohol"
Disposable tips (filter tips and laboratory plastic RNA-se / DNA-se Free)
Pipettes
High speed centrifuge for small tubes
Vortex
Incubation oven at 70 ° C
Personal protective equipment (e.g. lab coat, gloves, glasses)

Viral Nucleic Acid Purification Protocol

Before starting the protocol, prepare the following:

Table 3.1: Preparation of working solutions.

Component	50 samples
Lysis buffer	Add 1 ml Lysis Buffer to the RNA vial with a carrier, pipette the solution back into the vial
Wash buffer 2 (concentrate)	12 ml Add 48 ml ethanol (96-100%)
Proteinase K	30 mg Add 1.35 ml Proteinase Buffer

Storage and stability

Store the kit at room temperature (18-25 ° C) until delivery.
Lyophilized Proteinase K can be stored at room temperature (18-25 ° C) before the expiration date without deterioration in quality. Before first use kit, add the indicated volume of Proteinase Buffer to dissolve Lyophilized Proteinase K Dissolved Proteinase K should be stored at -20 ° C up to 6 months, but no longer than the established expiration date. Discrete use test kits are recommended to dispense Proteinase K into aliquots.
Lyophilized carrier RNA can be stored at room temperature (18-25 ° C) before the expiration date without deterioration. Add 1 ml Lysis buffer to the carrier RNA vial before first use.
Dissolve carrier RNA and pipette the solution back into the vial. RNA transporter has a limited shelf life in the Lysis buffer. Transfer RNA Buffer can be stored at 4 ° C for up to 4 weeks; at -20 ° C the dissolved carrier RNA is stable for one year. Storage at 4 ° C or below may cause precipitation salts. If there is visible sediment, dissolve it before use heating to 40-60 ° C for no more than 5 minutes. Do not heat the Lysis Buffer containing RNA transporter more than 4 times! Frequent heating, temperature> 80 ° C, continuous thermostating will accelerate the degradation of the carrier RNA.
Wash Buffer 2: Add the indicated volume (see table above or on vial) ethanol (96-100%) to concentrated Wash Buffer 2. Mark on label that ethanol has already been added. Store wash buffer 2 at room temperature. temperature. Under these conditions, Wash Buffer2 can be stored for up to one year, but only before the expiration date.

Isolation of viral DNA

Step	Remarks and comments
1. Add 600 μl Lysis Buffer containing the RNA-transporter into a test tube for samples contained in the kit.	
2. Place 150 μL of sample in a tube for samples containing lysis buffer and RNA transporter.	
3. Add 20 µl Proteinase K solution to sample tube.	Proteinase K is required for DNA lysis viruses. Only add Proteinase K after after Lysis Buffer 1 and the sample are already are in a test tube.

Step	Remarks and comments
4. Pipette the resulting mixture up and down and Vortex thoroughly.	Make sure the mixture is at least at least 1 minute at room temperature before thermostating.
5. Incubate 5 minutes at 70 ° C.	
6. Slightly centrifuge test tube for samples (~ 1 sec at 2,000 x g) to remove drops from the lid	For a short time
7. Add 10 μl of VKO	
8. Add 600 µL ethanol (96-100%) to clear the lysate.	Vortex for 10 - 15 sec
9. Carefully load 680 µL of lysate onto the column filter, place it in the NK collection tube and close the cover.	Centrifuge 1 min at 8,000 xg
10. Place the filter column in a new tube for NK selection (2 ml, attached) and get rid of from a test tube with a liquid obtained at the previous stage.	
11. Load the remaining lysate (about 680 µL) onto the filter column, and close the lid.	Centrifuge 1 min at 8,000 xg
12. Place the filter column into a new tube for NK selection (2 ml, attached) and get rid of from a test tube with a liquid obtained at the previous stage.	
13. Add 500 μl of Wash Buffer 1 to column for filtration.	Centrifuge 1 min at 8,000 xg
14. Place the filter column into a new tube. for NK selection (2 ml, attached) and get rid of from a test tube with a liquid obtained at the previous stage.	
15. Add 600 μL of Wash Buffer2 to column for filtration.	Centrifuge 1 min at 8,000 xg
16. Place the filtration column in a new collection tube (2 ml, included) and	

get rid of from test tubes from liquid obtained in the previous step.

17. Add 200 μl of Wash Buffer 2 to column for filtration. — Centrifuge 3 min at 11,000 xg

18. Place the filtration column in the tube for elution (1.5 ml) and get rid of test tubes with the liquid obtained before.

19. Add 50 µl of Eluent (previously heated to 70 ° C) and incubate for 1-2 min at 70 ° C. — Centrifuge 1 min at 11,000 xg

Isolation of DNA from a tissue sample, meat products, whole blood (columnar method).

Carried out using the kit for the isolation of nucleic acids (NK) included in composition of the test system.

Table 2.2: Kit components.

Name	amount for 50 samples	Description
Lysis buffer 1	20 ml	
Lysis buffer 2	15 ml	
Wash buffer 1	30 ml	
Wash buffer 2 (concentrate)	12 ml	
Eluent	13 ml	pH 8.5
Proteinase buffer	1.8 ml	
Proteinase K	30 mg	Lyophilized
Column filter	50	Green rings
NDT sampling tubes	2x50	Collection tubes (2 ml) included
Sample tubes	50	Sample tubes (2.0 ml) containing stabilized internal controls included with the test system

Required reagents and equipment

96-100% ethanol (to improve nucleic acid precipitation and to preparation of wash buffer 2)
Disposable tips (filter tips and laboratory plastic RNA-se / DNA-se Free)
Pipettes
High speed centrifuge for small tubes
Vortex
Incubation oven at 70 ° C
Personal protective equipment (e.g. lab coat, gloves, glasses)

Viral Nucleic Acid Purification Protocol

Before starting the protocol, prepare the following:

Table 3.2: Preparation of working solutions.

Component	50 samples
Wash buffer 2 (concentrate)	12 ml Add 48 ml ethanol (96-100%)
Proteinase K	30 mg Add 1.35 ml Proteinase Buffer

Storage and stability

Store the kit at room temperature (18-25 ° C) until delivery.
Lyophilized Proteinase K can be stored at room temperature (18-25 ° C) before the expiration date without deterioration in quality. Before first use

kit, add the indicated volume of Proteinase Buffer to dissolve Lyophilized Proteinase K Dissolved Proteinase K should be stored at -20 ° C up to 6 months, but no longer than the established expiration date. Discrete use test kits are recommended to dispense Proteinase K into aliquots.
Storage at 4 ° C Lysis Buffers 1 and 2 or below may cause precipitation salts. If there is visible sediment, dissolve it before use heating to 50-70 ° C.
Wash Buffer 2: Add the indicated volume (see table above or on vial) ethanol (96-100%) to concentrated Wash Buffer 2. Mark on label that ethanol has already been added. Store wash buffer 2 at room temperature. temperature. Under these conditions, Wash Buffer2 can be stored for up to one year, but only before the expiration date.

Isolation of viral DNA

Step	Remarks and comments
1. Samples of pathological material (spleen, kidney, lungs, lymph nodes, etc.) should be crushed by special mechanical homogenizers (25 mg of tissue is placed in add 250 µl FBI to Eppendorf tube or saline and homogenize). Centrifuge the resulting suspension at 11000 g. 25 mg pork food origin (sausage, sausages) place in test tube Eppendorf, add 250 µl of purified water, shake on a vortex and warm in for 5 min at 65 ° C. Centrifuge at 11000 g.	When sample preparation food pork products allowed use mechanical homogenizers. (25 mg put the product in a test tube Eppendorf add 250 µl PBS or saline and homogenize). Received suspension centri-Fug at 11000 g.
2. Place 60 μl of the supernatant in microtube (from set not supplied)	
3. Add 180 μL of Lyse to the sample. buffer 1 and 25 µl Proteinase K. Shake well.	If several samples allowed in advance mix Lysis Buffer 1 and Proteinase K and add immediately to samples.
4. Incubate at 56 ° C for 10 minutes with constant stirring.	
5. Stir on the vortex, centrifuge and place the sample in sample tube.	If the sample contains visible particles - centrifuge at 11000 g and supernatant transfer to sample vial.
6. Add 200 μl Lysis Buffer 2 to sample tube.	
7. Vortex thoroughly 10 - 15 sec	

8. Incubate for 10 minutes at 70 ° C.

9. Vortex for 10 - 15 seconds

10. Lightly centrifuge the tube to vortex samples to remove drops off the lid — For a short time

11. Add 10 μl VKO

12. Add 210 μL ethanol (96-100%) and Vortex for 10 - 15 sec — After adding ethanol can to form a precipitate. It will not affect for completeness of DNA extraction. Track down so that all precipitate is transferred to column

13. Load the lysate carefully onto the column. filter, place it in a test tube for sampling NK and close the lid. — Centrifuge for 1 min at 11,000 xg

14. Place the filter column in a new test tube for selection NK (2 ml, attached) and dispose of the tube with

liquid obtained in the previous stage.

15. Add 500 μl of Wash Buffer 1 per column for filtration. — Centrifuge for 1 min at 11,000 xg

16. Get rid of from flowing liquids, obtained in the previous step. Return filter column into a test tube for NK sampling.

17. Add 600 μl of Wash Buffer2 per column for filtration. — Centrifuge for 1 min at 11,000 xg

18. Get rid of from flowing liquids, obtained in the previous step. Return filter column into a test tube for NK sampling.

19. Remove the remaining amount of ethanol. — Centrifuge for 3 min at 11,000 xg

20. Place the filtration column in elution tube (1.5 ml not included with the kit) and get rid of test tubes with liquid received before this.

21. Add 60 μl of Eluent and incubate 1-2 min at room temperature — Centrifuge for 1 min at 11,000 xg

22. The isolated DNA can be stored in deep freeze conditions (-20 ... -80 ° C) for several months; allowed storage at + 2 ... + 8 during the day.

Isolation of DNA from a tissue sample, meat products, whole blood by sorbent method

Carried out using the kit for the isolation of nucleic acids (NK) included in composition of the test system.

Table 2.3: Kit components.

Name	Quantity for 50 ml samples
Lysis solution	7.5
Sorbent	1.0
Washing solution No. 1	10.0
Washing solution No. 2	10.0
Washing solution No. 3	10.0
Elution solution	5.0

Required reagents and equipment

Disposable tips (filter tips and laboratory plastic RNA-se / DNA-se Free)
Pipettes
High speed centrifuge for small tubes
Vortex
Incubation oven at 70 ° C
Personal protective equipment (e.g. lab coat, gloves, glasses)
Medical aspirator

Storage and stability

The isolation kit should be stored at 2-8 ° C throughout

expiration date. After opening the package, the lysis solution and rinsing solution No. 1 should be stored in a dark place. The shelf life of the kit is 6 months.

Isolation of viral DNA

Step

1. Samples of pathological material (spleen, kidneys, lungs, lymph nodes, etc.) should be crushed with special mechanical homogenizers (place 25 mg of tissue in a test tube Eppendorf add 250 µl PBS or saline and homogenize). The resulting suspension centrifuge at 11000 g.
   Place 25 mg of a food product of pig origin (sausage, sausages) in a test tube Eppendorf, add 250 µl of purified water, shake on a vortex and warm up for 5 min at 65 ° C. Centrifuge at 11000 g.

2. Pipette 60 µl of the prepared biomaterial into the test tubes for the studied samples. IN biomaterial is not added to the test tube "K-".

3. Into the tube marked "K-" add 60 µl of sterile saline.

4. Pipette 10 µL of ICO into each tube.

5. Prepare a mixture of lysis solution and sorbent. Mix in a separate tube: 150 x (N + 1) µl of lysis solution, 20 x (N + 1) previously resuspended sorbent,

where N + 1 - the number of analyzed samples, taking into account "K-" (N) with a margin of 1 sample.

6. Add 170 µl of this mixture to each tube.

7. Close the tube lids tightly, vortex for 3-5 seconds.

8. Incubate the tubes for 15 min at 50 ° C.

9. Centrifuge the tubes at 11000 g for 1 min.

10. Without touching the sediment, completely remove the supernatant liquid (with a separate tip from each tube)

11. Add 200 µl of wash solution # 1 to the sediment and shake the tubes on a vortex for 3-5 sec.

12. Centrifuge the tubes at 11000 g for 1 min.

13. Without touching the sediment, completely remove the supernatant liquid (with a separate tip from each tube).

14. Add 200 µl of wash solution # 2 to the sediment and shake the tubes on a vortex for 3-5 sec.

15. Centrifuge the tubes at 11000 g for 1 min.

16. Without touching the sediment, completely remove the supernatant liquid (with a separate tip from each tube).

17. Add 200 µl of washing solution # 3 to the sediment and vortex the tubes for for 3-5 sec.

18. Centrifuge the tubes at 11000 g for 1 min.

19. Without touching the sediment, completely remove the supernatant liquid (with a separate tip from each tube).

20. Open the tube lids and dry the pellet at 50 ° C for 5 min.

21. Add 60 µl elution solution to the sediment and vortex the tubes for 5-10 sec.

22. Heat the tubes at 50 ° C for 5 minutes.

23. Centrifuge the tubes at 11000 g for 1 min. If the sample is to be stored more than 7 days, transfer the supernatant to a new tube.

The supernatant containing the isolated DNA is ready for addition to the reaction mixture for PCR amplification.
The resulting DNA preparation can be stored for up to 5 days at a temperature of 2–8 ° C. More than 5 days the DNA preparation should be stored at minus 20 ° C. Shelf life - up to 6 months

How the ASFV kit works

The ASFV test kit is a polymerase chain assay based real-time reactions (Real-Time PCR). The kit is designed for quality detection ASFV transcripts . The qualitative standard consists of synthetic DNA ASFVs to be amplified with the analyzed samples.

Figure 1: A kit for determining the ASFV : an overview of the protocol of the experiment. Reagents and consumables required for Steps 1-2 are included in the kit.

1. Perform selection total DNA from serum and blood plasma, s using recommended set for extraction

Select according to manufacturer's instructions for the kit extraction

2. Cook 25 × ASFV_oligonucleotide mixture

Add 35 µl purified water, vortex for 3 seconds.

5 sec, 10,000g

Add 20 µL 1 × Reagent Mix to all test tubes with test samples and test tubes with concentration standards.

Add 5 µl of DNA samples to the tubes for

3. Prepare and pour by test tubes 1 × working reagent mixture solution, add samples		test samples. Add 5 µl of purified water to contamination control tubes, and 5 µl of each of the dissolved standards concentration in standard tubes quantitative determination. 5 sec, 1,500g
4. Close the tubes, install them in thermal cycler, set up and run RT-PCR		
5. Data analysis **Analysis of growth curves in tested samples, control samples and quantitative definitions**	DNA concentration growth curves	Growth curves of internal controls samples

Universal protocol for devices supporting FAM detection or FAM / HEX fluorescence

The described examples are given for the Real-Time PCR system RotorGene and BioRad CFX96 TM

Important! Include at least one positive sample in your sample. ASFV DNA control, negative control Water for PCR studies, and also controls contamination of reagents containing instead of the investigated water samples for PCR studies. The control sample is included in this set. Negative and contamination controls, in where amplification has occurred indicate sample contamination exogenous genetic material. In this case, you need to repeat RT-PCR using freshly prepared reagents and samples needed investigate at least two (preferably three) times in a row.

In order to maintain the full activity of Taq polymerase, all operations for dosing and mixing of reagents must be carried out on ice or on special cooling surface !!

Table 4. Stages of work

Stage of work	Remarks and comments
Preparation of standards	
1. Thaw the quality standard before room temperature. Shake and centrifuge on centrifuge-vortex.	When working with subjects samples and standard samples always wear gloves.

2. Preparation of 25 × concentrate of the reagent mixture from lyophilized ASFV oligonucleotide mixed. Centrifuge tubes with oligo mixt on vortex centrifuge before opening them to lyophilized oligonucleotide powder guaranteed to move from the walls of the tubes to the bottom and did not fly apart when the tube was opened. Add 35 µl purified water in a vial containing lyophilized oligo mixed. Close the bottle and vortex for 3 seconds, then centrifuge for 5 seconds on a centrifuge vortex.

Dissolved reagent mixture can be stored at + 2-8 ° C in protected from light place for minimum 3 months (not freeze!).

3. Preparation of a working solution of the basic reagent mixtures (**Table 5**). Counting the total number of samples, subjected to PCR (test samples + all control samples). Vortex the prepared mixture for 3 seconds, then centrifuge for 5 seconds on a vortex centrifuge.

We recommend adding to the calculation 1-2 additional samples to compensate inaccuracies at dosing and losses at pipetting.

All the above manipulations are performed **on ice!**

Table 5: List and volumes of reagents for preparation of the basic working solution reagent mixture. Vortex the finished mixture and precipitate the drops with lids on a vortex centrifuge.

Reagent	**No ROX**	**High concentration ROX (1 ×)** 1)	**Low concentration ROX (0.1 ×)** 2)	
	µl in 1 sample	**µl in 1 sample**	**µl in 1 sample**	**The final concentration**
Water for PCR-research	16.1	15.6	16.05	-
Buffer mix2	2.5	2.5	2.5	1 ×
ASFV-oligonucleotide mixed	1.0	1.0	1.0	1 ×
Taq polymerase (5 U / µl)	0.4	0.4	0.4	2.0 U per 1 sample
50 × ROX	-	0.5	0.05 3)	1 × / 0.1 ×

1) In working with a family of thermocyclers PRISM ABI ® 7000 / 7300/7700 (Thermo Fisher Scientific)
2) To use a family of thermocyclers 7500 Real Time PCR Fast System, StepOne ® (Thermo Fisher Scientific)
3) If the number of PCR samples is small, pre-dilute 50 × ROX concentrate with water for PCR studies in a ratio of 1:10, then add 0.5 µl of the resulting solution to every sample. In this case, 18.6 µL of PCR water must be added to each sample.

research.

Stage of work	Remarks and comments
4. Prepare enough tubes for amplification of controls (OKO, K +, K-) and subjects samples, place them in a refrigerated rack.	When working with samples and Always wear gloves.
5. Add 20 μl of stock working solution reagent mixture (**see table. 5**) in all samples.	
6. Transfer 5 μL of PCR water to the Negative control tubes are not containing samples (so-called contamination controls - K-) and 5 μl of the standard (K +) into a test tube with positive control.	The final volume of the reaction mixture should not exceed 25 μl.
7. Add 5 μL of DNA samples to the appropriate test tubes with test samples.	The total volume of the reaction mixture is not should exceed 25 μL.
8. Close the sample tubes with the appropriate caps, cap strips or optical film.	
9. Reset liquid on the bottom test tubes centrifugation on the vortex centrifuge in within 5 seconds.	
10. Carefully place the prepared tubes into thermal cycler, close the lid.	

BIO RAD CFX 96

11. Run the PCR protocol setup: a. Turn on the instrument by pressing the power button, then start the CFX Manager software. b. To create a new protocol select "Create New" ("Create new"): the protocol editor will open; in otherwise, select "Select Existing" existing "): the file manager will start, where you can choose to use or editing an existing log file. Can also use the "Express Load" dropdown menu ("Express download").	If you are using PCR-equipment, different from described, strictly follow manufacturer's recommendations, taking taking into account the differences in the settings of your tool.
c. Protocol editor Create new protocol or run existing **.** Select any stage of the protocol on graphic or text screen. Click Add plate read to Step ”	

tablets ") to set the moment of data reading.
fluorescence. Set the moment to read data from
plates **in the annealing / elongation step** at 59 ° C.
Click on the number of repetitions (stage "GOTO"),
set the number of cycles in the loop to 45.

12. Click on the “Setup Plate” tab.
tablets ").
a. Click “Create New” to
open the tablet editor and create a new circuit
experiment. Otherwise, click on "Select Existing"
("Select existing") to start the file
manager and select an existing schema file
experiment to use or edit.
b. Using the "Express Load" dropdown menu
("Express download"), select the default file
"QuickPlate_96wells_AllChannels.pltd".
c. Click the "Start Run" tab to
start PCR with the existing experimental design.

13. Tablet editor: used to create a new
schemes experiment or editing
existing.
a. Using the tablet editor toolbar,
indicate the mode of data collection that you plan
use.
b. Click on the "Select Fluorophores" button.
fluorochromes ") and indicate what in given
the experiment needs to use FAM (viral DNA)
and HEX (internal control).
c. On the tablet diagram, indicate the cells to be used.
d. select "Sample Type" from the drop-down menu.
samples ").
e. Select the required checkbox (or several),
to set the dyes corresponding to the selected
cells.
f. Type in every cell name
the corresponding sample, press "Enter".

Rotor-Gene

14. Create new protocol or run

existing.

a. Press the New button in the program menu
b. Select the type of rotor. Check No Domned 0.2 ml
c. Select the volume of the reaction mixture 25 µl
d. Set amplification parameters in accordance with **Table 6** .
e. Fluorescence measurement on FAM (Green) and HEX (Yellow) is carried out at 59 ° C

15. Set up the detection channels according to Table 7

16. Start amplification with the Start run button

Table 6: Amplification Conditions for Quantification of ASFV DNA (universal for all thermal cyclers)

Stage	Temperature	Time	Number of repetitions
Taq polymerase activation	95 ° C	4:00 minutes	1
Melting (denaturation)	95 ° C	5 sec	45
Annealing / Elongation / Detection fluorescent signal (FAM / HEX)	59 ° C	30 sec	

The total time required to complete RT-PCR with this protocol is approximately 2 hours 00 minutes.

Table 7: Configuring the detection channel and other equipment parameters

RT-PCR thermal cycler	Channel detection	Other equipment parameters
		No-ROX Rotor-Gene 3000: Dynamic tube: Yes Slope correction: Yes Ignore first: 4 No template control threshold: 5%

Rotor-Gene ™ 3000/6000, Rotor-Gene ™ Q (Qiagen)	Green (Green) Yellow (Yellow)	Perform Calibration / Optimization Before 1th Acquisition - Min Reading 5Fl - Max Reading 10Fl Threshold modus: manual ASFV: 0.01-0.04, exponential amplification curve, above background values, aiming for the minimum value Rotor-Gene 6000 / Q: Dynamic tube: Yes Slope correction: Yes Ignore first: 1 No template control threshold: 5% Perform Calibration / Optimization Before 1th Acquisition - Min Reading 5Fl - Max Reading 10Fl Threshold modus: manual ASFV: 0.01-0.04, exponential amplification curve, above background values, aiming for the minimum value
CFX96TM; iCycler IQ ™ ; IQ5, MiniOpticon ™ , CFX Connect ™ Real-Time PCR Detection System (Bio-Rad)	FAM	CFX96: Need to manually set the threshold value in 50-250 relative units fluorescence (RFU).
SmartCycler II ® (Cepheid)	FAM (channel 1)	not
Mx3000P, Mx3005P (Agilent / Stratagene)	FAM / SYBR	Check that no reference has been set. dye
LightCycler ® 480II, LightCycler ® 96 (Roche)	FAM	not
Mastercycler ® ep realplex (Eppendorf)	FAM	not
High / low concentration of passive ROX		
7500 Fast Real Time PCR System	FAM	Select « *auto baseline»* («automatic baseline "), manually set the threshold value ≥0.02
ABI PRISM ® 7000/7300/7700 Sequence Detection System, StepOne ® (Thermo Fisher Scientific)	FAM	Select " *auto baseline*" ("auto base level"), manually install threshold value> 0.05

Data analysis

Each cycle of DNA amplification results in the generation of a fluorescent signal, measured in the Green (**FAM**) channel for the **target** and Yellow (**HEX**) for the **internal control** , which leads to the formation of a sigmoid growth curve (in logarithmic scale). Analyze the data as recommended equipment manufacturer (see, for example, the thermal cycler manual Rotor-Gene 6000 / Q or CFX96 TM Real Time PCR Detection System, Bio-Rad) using appropriate software and taking into account the recommendations given in **Table 7** (as well as the following notes). Determination of ASFV DNA in Subjects samples based on C_T (threshold cycle) growth curves obtained at amplification of the tested DNA samples.

1. When the C_T value on the FAM channel is less than or equal to 40, and the C_T value on the HEX channel less than 40 sample contains fragments of the ASFV genome (positive).
2. If the C_T value on the FAM channel is more than 40 or its absence (N / A) and the C_T value on

In the HEX channel less than 40, the sample does not contain fragments of the ASFV genome (negative).

3. If the C_T value on the FAM channel is less than or equal to 20, and there is no C_T value on the channel HEX (N / A), the sample contains fragments of the ASFV genome (positive).
4. If the C_T value on the FAM channel is more than 20, and there is no C_T value on the HEX channel (N / A) it is necessary to repeat the study of the sample, including two tenfold dilutions. If, at the same time, one of the samples shows sigmoid curves along the channel FAM and HEX - the sample contains fragments of the ASFV genome (positive).
5. If there is no C_T value on the FAM (N / A) channel, and there is no C_T value on the channel HEX (N / A) the result is considered invalid. Research needs to be repeated sample (n = 2), including the extraction step.
6. For a reliable interpretation of the results, take into account the kinetic curves accumulation of fluorescence only in the sigmoidal form.

Interpretation	C_T value by channel FAM	C_T value by channel HEX
Positive (+)	≤ 40	≤40
Negative (-)	N / A	≤40
Positive (+)	≤ 20	N / A
Repeat study, including breeding	≥20	N / A
Repeat research (n = 2)	N / A	N / A

In case of dubious positive results, it is possible contamination of tools and workplace - decontaminate laboratory activities.

The set threshold level of detection can significantly affect the values The C_T . Set threshold levels according to the recommendations from **Tables 7** .

Solution of problems

Table 8: Possible causes of errors and how to fix them

Problem	**Possible reason Problems**	**Decision**
Sample preparation problems		
Insufficient degree purity of extracted DNA or synthesized DNA	RNA / DNA sample contamination protein salts, carbohydrates and other organic matter, inhibitory PCR	Avoid phenolic and / or other extraction methods, use only supplied nucleic acid extraction kit acids.
Problems with pipetting		
Received fluorescent		Repeat extraction and / or PCR with

signal from samples, not containing ASFV DNA , and / or from controls contamination of reagents	Contamination of negative samples amplicons ASFV	new reagents; conduct decontamination of instruments and workplace.
Total volume reaction mixture different from 25 µl	Pipetting errors for example skipping or re-fill cells	Use multichannel pipettes, automated pipetting, or develop attention and concentration.
	Amplification problems	
Unusually large C_T standard values and / or overpriced ASFV DNA concentration in test samples	Invalid protocol amplification	Check your settings equipment, follow instructions from the manual use of the device.
	Violation of conditions and / or terms storage of reagents	Check storage conditions and shelf life suitability.
	Decay of detectable DNA	Use consumables and reagents not containing nucleases; immediately after synthesis place DNA samples on ice.
Non-sigmoid shape growth curves of standards concentration and subjects samples	Frequent defrosting / thawing or wrong storage of a mixture of dissolved reagents	Read the instructions, check storage conditions, prepare fresh reagent mixture.
	Storage conditions are not correspond to the recommended expired kit	Check storage conditions and shelf life suitability.
	Fluorescence measurement the signal is disabled; camera incorrectly installed	Check your settings equipment.
Is absent fluorescent signal	Wrong channel selected fluorescent signal recording	For ASFV DNA determination select the FAM channel. For definitions of internal control select the HEX channel.
	Invalid protocol amplification	Check your settings equipment.
	Violation of conditions and / or terms storage set	Check storage conditions and shelf life suitability.

Different kind amplification of ASFV DNA in tested samples, unparalleled growth curves in exponential reaction phase	An excess of PCR inhibitors in sample	Use the recommended extraction set, exactly follow instructions manufacturer. DNA dilution before analysis can reduce content of inhibitors in the sample (see protocol, step 7).
	Incorrectly assembled material (e.g. heparinized blood)	Use correctly assembled samples.
	Dirty optical lenses	See the care section of the instructions for use of appropriate thermal cycler; if it allows construction - once a month wipe the lenses using

		absolute ethyl alcohol and cotton buds.
Registered low fluorescent level signal during amplification detectable DNA	Cooling system contamination and / or optical matrices sensor	See the care section of the instructions for use of appropriate thermal cycler; can also fill each cell of the sensor isopropanol, incubate 10 minutes at 50 ° C, remove isopropanol and rinse bidistilled water.
	Evaporation from the cell during PCR	Covers for tablets, test tubes, strips should be maximum effective.
	If the tool requires use ROX passive dye - using the wrong ROX concentration	Use ROX solution optimal concentration, supplied with this kit; make sure to use "AR" version of the set.

ТЕСТ-СИСТЕМА ДЛЯ ОБНАРУЖЕНИЯ ДНК ВИРУСА ASFV МЕТОДОМ REAL-TIME PCR
ТУ BY 391360704.011-2015
ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ.

	Предназначен для 100/50 качественных определений ДНК ASFV (перечислены все варианты поставки)	
	Универсальный формат Предназначен для использования с блочными и карусельными термоциклерами, способными работать с пробами объёмом 25 мкл и регистрировать FAM/HEX флуоресценцию, например, CFX96^{TM}, Rotor-Gene	ТУ BY 391360704.011-2015 (100 тестов) / (50 тестов)
- 20°C - 30°C	Рекомендованная температура хранения	
	ООО «СИВитал» 210017, Республика Беларусь, г. Витебск, ул. Гагарина, 11 к. 12	Tel.: +375-212-23-20-07 Fax: +375-212-23-14-48 E-mail: info@sivital.by

Содержимое тест системы

Таблица 1: Компоненты тест-системы

Маркировка	Упаковка (50 тестов)	Упаковка (100 тестов)	Вид упаковки	Описание
Комплект для выделения НК	50	100	Коробка/пакет с компонентами набора	Набор для извлечения нуклеиновых кислот из биологического материала
ASFV-Олиго-нуклеотидный микст	2 флакона	3 флакона	янтарный флакон, янтарная крышка	Лиофилизированная смесь реагентов, содержащая специфические праймеры *ASFV*, ДНК-зонды, смесь дезоксирибонуклеотидов, а также олигонуклеотиды
ASFV-стандарт	2 стандарта	4 стандарта	–	Пробирки со стандартами концентрации, содержащие синтетическую ДНК *ASFV*
Вода для PCR-исследований (ОКО)	2×1.5 мл	4×1.5 мл	бесцветный флакон, бесцветная крышка	Очищенная вода для ПЦР
50× ROX	1×0.20 мл	1×0.20 мл	янтарный флакон, фиолетовая крышка	50× концентрат пассивного референсного красителя ROX
Taq-полимераза	1×135 Ед	2×135 Ед	бесцветный флакон, бесцветная крышка	Фермент Taq-полимераза (5 Ед/мкл)
Буферная смесь2	1×0.5 мл	2×0.5 мл	бесцветный флакон, оранжевая крышка	10× концентрат ПЦР-буфера, содержащий хлорид магния
ВКО	2×0.3 мл	4×0.3 мл	–	Пробирки с внутренними стандартами, содержащие синтетическую ДНК
	1	1	Инструкция	

Условия хранения, стабильность

Набор для обнаружения ASFV может транспортироваться при комнатной температуре, за исключением Taq-полимеразы и 10× концентрата ПЦР-буфера, которые должны перевозиться на сухом льду. Хранить весь набор (включая Taq-полимеразу и 10× ПЦР-буфер) необходимо в темном месте при –20…–30°С, куда его следует поместить сразу после доставки. Стабильность набора гарантируется в течение всего срока хранения (при хранении в оговоренных выше условиях). Реагенты, не включенные в набор, должны храниться в условиях, рекомендованных их производителями.

Замечание. Необходимое количество реагентной смеси (ASFV-олигонуклеотидный микст) непосредственно перед употреблением необходимо растворить в воде для PCR-исследований. Остаток растворённой реагентной смеси может храниться при +2+8°С в течение по крайней мере 3 месяцев (не замораживать! всегда беречь от попадания света!)

Ограничения в использовании продукта

Очень высокая концентрация гепарина в образце может привести к критическому снижению коэффициента извлечения копий ДНК ASFV. При использовании образцов иной природы возможно получение неправильных результатов. Тест-система может быть использована только с оговоренным ПЦР-оборудованием.

Сбор и хранение образцов

Сбор и обработка клинических образцов

Кровь (плазма, сыворотка)

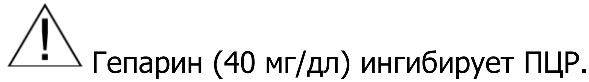
- Соберите 5-10 мл периферической крови, используя стандартные пробирки для сбора образцов.
- Необходимо использовать пробирки для сбора крови, покрытые антикоагулянтом ЭДТА или цитратом.
- Допускается отобрать кровь в стандартные пробирки с предварительно добавленными 3% растворами вышеуказанных коагулянтов из расчета 10:1.
- Центрифугировать кровь при 1500 об/мин в течение 10 мин (получение плазмы).
- Для получения сыворотки крови забор кровь проводят без антикоагулянта

Плотные ткани

- Гомогенизируйте 25 мг ткани в присутствии 250 мкл физраствора или фосфатного буфера.
- Выделенное ДНК может храниться в условиях глубокой заморозки (–20…–80°С) в течение нескольких месяцев; допускается хранение при +2…+8 в течение суток.

Химические взаимодействия

- Повышенный уровень билирубина, липидов, гемоглобина, ЭДТА (≤30 мМ) и цитрата (≤3.13%) не влияют на работу тест-системы.


Гепарин (40 мг/дл) ингибирует ПЦР.

Реагенты и оборудование, предоставляемые пользователем

- Термоциклер для Real-Time PCR.
- Программное обеспечение термоциклера для анализа и протоколирования данных.

- Пластиковые расходные материалы для термоциклера (RNA-se/DNA-se Free).
- Пипетки, стерильные наконечники для них с аэрозольным барьером (RNA-se/DNA-se Free).
- Микроцентрифуга скоростная, пригодная для пробирок объёмом 0.2 мл, 1.5 мл и 2.0 мл, а также для 96-луночных планшет.
- Твердотельный термостат, пригодный для пробирок объёмом 0.2 мл, 1.5 мл и 2.0 мл, а также для 96-луночных планшет.
- Вортекс.
- Пробирки 1,5 мл.
- Перчатки, лабораторный халат.

Выделение ДНК из образца сыворотки и плазмы крови (колоночный метод).

Осуществляют с помощью комплекта для выделения нуклеиновых кислот (НК), входящего в состав тест-системы.

Таблица 2.1: Компоненты комплекта.

Наименование	Количество на 50 проб	Описание
Лизирующий буфер	35 мл	
Промывочный буфер 1	30 мл	
Промывочный буфер 2 (концентрат)	12 мл	
Вода для PCR-исследований	13 мл	pH около 7 – 8
Элюент	13 мл	pH 8.5
РНК-переносчик	1 мг	Лиофилизировано
Протеиназный буфер	1.8 мл	
Протеиназа К	30 мг	Лиофилизировано
Колонка фильтр	50	Тёмно-синие кольца
Пробирки для отбора НК	4x50	Пробирки для сбора (2 мл) прилагаются
Пробирки для образцов	50	Пробирки для образцов (2.0 мл) содержащие стабилизированные внутренние контроли прилагаются с тест-системой
Пробирки для элюирования	50	Пробирки для элюирования (1.5 мл)

Необходимые реагенты и оборудование

- 96-100 % этанол (для улучшения осаждения нуклеиновых кислот и для приготовления промывочного буфера 2). Только марки «медицинский спирт»
- Одноразовые наконечники (рекомендуются наконечники с фильтром и лабораторный пластик RNA-se/DNA-se Free)
- Пипетки
- Центрифуга для маленьких пробирок высокоскоростная
- Вортекс
- Термостат для инкубации при 70 °С
- Средства личной защиты (например, лабораторный халат, перчатки, очки)

Протокол очистки вирусных нуклеиновых кислот

Перед началом протокола приготовьте следующее:

Таблица 3.1: Приготовление рабочих растворов.

Компонент	50 проб
Лизирующий буфер	Добавьте 1 мл Лизирующего буфера во флакон с РНК-переносчиком, пипетируйте раствор обратно во флакон
Промывочный буфер 2 (концентрат)	12 мл Добавьте 48 мл этанола (96-100 %)
Протеиназа К	30 мг Добавьте 1.35 мл Протеиназного буфера

Хранение и стабильность

До момента доставки необходимо хранить набор при комнатной температуре (18-25 °С). Лиофилизированная Протеиназа К может храниться при комнатной температуре (18-25 °С) до истечения срока годности без ухудшения качества. Перед первым использованием набора, добавьте указанный объём Протеиназного буфера, чтобы растворить лиофилизированную Протеиназу К. Растворенную Протеиназу К следует хранить при -20 °С до 6 месяцев, но не дольше установленного срока годности. При дискретном использовании тест-системы рекомендуется Протеиназу К разлить на аликвоты.
Лиофилизированный РНК-переносчик может храниться при комнатной температуре (18-25 °С) до истечения срока годности без ухудшения качества. Добавьте 1 мл Лизирующего буфера к флакону с РНК-переносчиком перед первым использованием. Растворите РНК-переносчик и пипетируйте раствор обратно во флакон. РНК-переносчик имеет ограниченный срок хранения в Лизирующем буфере. Буфер с РНК-переносчиком может храниться при 4°С до 4 недель; при -20°С растворенный РНК-переносчик стабилен на протяжении одного года. Хранение при 4 °С или ниже может вызывать преципитацию солей. Если присутствует видимый осадок, перед использованием следует его растворить нагреванием до 40-60 °С не более 5 минут. Не нагревайте Лизирующий буфер, содержащий РНК-переносчик более 4 раз! Частое нагревание, температура >80 °С, продолжительное термостатирование ускорят деградацию РНК-переносчика.
Промывочный буфер 2: Добавьте указанный объём (смотрите таблицу выше или на флаконе) этанола (96-100 %) к концентрированному Промывочному буферу 2. Отметьте на этикетке, что этанол уже добавлен. Храните промывочный буфер 2 при комнатной температуре. В этих условиях Промывочный буфер2 может храниться до одного года, но только до истечения срока годности.

Выделение вирусной ДНК

Шаг	***Замечания и комментарии***
1. Добавьте 600 мкл Лизирующего буфера содержащего РНК-переносчик в пробирку для образцов, содержащихся в наборе.	
2. Поместите 150 мкл образца в пробирку для образцов содержащую лизирующий буфер и РНК- переносчик.	
3. Добавьте 20 мкл раствора Протеиназы К в пробирку для образцов.	*Протеиназа К необходима для лизиса ДНК вирусов. Добавляйте Протеиназу К только после того как Лизирующий буфер 1 и образец уже находятся в пробирке.*

4. Пипетируйте полученную смесь вверх и вниз и тщательно перемешайте её на вортексе.	*Проследите чтобы смесь находилась, по крайней мере, 1 минуту при комнатной температуре перед термостатированием.*
5. Инкубируйте 5 минут при 70 °С.	
6. Слегка центрифугируйте пробирку для образцов (~1 сек. при 2,000 x *g*) чтобы удалить капли с крышки	*Кратковременно*
7. Добавьте по 10 мкл ВКО	
8. Добавьте 600 мкл этанола (96-100 %) чтобы очистить лизат.	*Перемешать на вортексе 10 – 15 сек*
9. Осторожно загрузите 680 мкл лизата на колонку фильтр, поместите её в пробирку для отбора НК и закройте крышку.	*Центрифугировать 1 мин при 8,000 x g*
10. Поместите колонку фильтр в новую пробирку для отбора НК (2 мл, прилагается) и избавитесь от пробирки с жидкостью, полученной на предыдущем этапе.	
11. Загрузите оставшийся лизат (около 680 мкл) на колонку для фильтрации, и закройте крышку.	*Центрифугировать 1 мин при 8,000 x g*
12. Поместите колонку фильтр в новую пробирку для отбора НК (2 мл, прилагается) и избавитесь от пробирки с жидкостью, полученной на предыдущем этапе.	
13. Добавьте 500 мкл Промывочного буфера 1 на колонку для фильтрации.	*Центрифугировать 1 мин при 8,000 x g*
14. Поместите колонку фильтр в новую пробирку для отбора НК (2 мл, прилагается) и избавитесь от пробирки с жидкостью, полученной на предыдущем этапе.	
15. Добавьте 600 мкл Промывочного буфера2 на колонку для фильтрации.	*Центрифугировать 1 мин при 8,000 x g*
16. Поместите колонку для фильтрации в новую пробирку для сбора (2 мл, прилагается) и избавитесь от пробирки с жидкостью, полученной на предыдущем этапе.	
17. Добавьте 200 мкл Промывочного буфера 2 на колонку для фильтрации.	*Центрифугировать 3 мин при 11,000 x g*
18. Поместите колонку для фильтрации в пробирку для элюирования (1.5 мл) и избавитесь от пробирки с жидкостью, полученной до этого.	
19. Добавьте 50 мкл Элюента (предварительно нагретого до 70 °С) и инкубируйте 1-2 мин при 70°С.	*Центрифугировать 1 мин при 11,000 x g*

Выделение ДНК из образца ткани, мясной продукции, цельной крови (колоночный метод).

Осуществляют с помощью комплекта для выделения нуклеиновых кислот (НК), входящего в состав тест-системы.

Таблица 2.2: Компоненты комплекта.

Наименование	Количество на 50 проб	Описание
Лизирующий буфер 1	20 мл	
Лизирующий буфер 2	15 мл	
Промывочный буфер 1	30 мл	
Промывочный буфер 2 (концентрат)	12 мл	
Элюент	13 мл	pH 8.5
Протеиназный буфер	1.8 мл	
Протеиназа К	30 мг	Лиофилизировано
Колонка фильтр	50	Зеленые кольца
Пробирки для отбора НК	2x50	Пробирки для сбора (2 мл) прилагаются
Пробирки для образцов	50	Пробирки для образцов (2.0 мл) содержащие стабилизированные внутренние контроли прилагаются с тест-системой

Необходимые реагенты и оборудование

- 96-100 % этанол (для улучшения осаждения нуклеиновых кислот и для приготовления промывочного буфера 2)
- Одноразовые наконечники (рекомендуются наконечники с фильтром и лабораторный пластик RNA-se/DNA-se Free)
- Пипетки
- Центрифуга для маленьких пробирок высокоскоростная
- Вортекс
- Термостат для инкубации при 70 °С
- Средства личной защиты (например, лабораторный халат, перчатки, очки)

Протокол очистки вирусных нуклеиновых кислот

Перед началом протокола приготовьте следующее:

Таблица 3.2: Приготовление рабочих растворов.

Компонент	50 проб
Промывочный буфер 2 (концентрат)	12 мл Добавьте 48 мл этанола (96-100 %)
Протеиназа К	30 мг Добавьте 1.35 мл Протеиназного буфера

Хранение и стабильность

До момента доставки необходимо хранить набор при комнатной температуре (18-25 °С).
Лиофилизированная Протеиназа К может храниться при комнатной температуре (18-25 °С) до истечения срока годности без ухудшения качества. Перед первым использованием

набора, добавьте указанный объём Протеиназного буфера, чтобы растворить лиофилизированную Протеиназу К. Растворенную Протеиназу К следует хранить при -20 °С до 6 месяцев, но не дольше установленного срока годности. При дискретном использовании тест-системы рекомендуется Протеиназу К разлить на аликвоты.

Хранение при 4 °С Лизирующего буфера 1 и 2 или ниже может вызывать преципитацию солей. Если присутствует видимый осадок, перед использованием следует его растворить нагреванием до 50-70 °С.

Промывочный буфер 2: Добавьте указанный объём (смотрите таблицу выше или на флаконе) этанола (96-100 %) к концентрированному Промывочному буферу 2. Отметьте на этикетке, что этанол уже добавлен. Храните промывочный буфер 2 при комнатной температуре. В этих условиях Промывочный буфер2 может храниться до одного года, но только до истечения срока годности.

Выделение вирусной ДНК

Шаг	***Замечания и комментарии***
1. Образцы патматериала (селезенка, почки, легкие, лимфоузлы и др.) должны быть измельчены специальными механическими гомогенизаторами (25 мг ткани поместить в пробирку Эппендорф добавить 250 мкл ФБР или физраствора и гомогенизировать). Полученную суспензию центрифугируйте при 11000 g. 25 мг пищевого продукта свиного происхождения (колбаса, сосиски) поместить в пробирку Эппендорф, добавить 250 мкл воды очищенной, встряхнуть на вортексе и прогреть в течение 5 мин при 65°С. Центрифугируйте при 11000 g.	*При пробоподготовке пищевых продуктов свиного происхождения допускается использовать механические гомогенизаторы. (25 мг продукта поместить в пробирку Эппендорф добавить 250 мкл ФБР или физраствора и гомогенизировать). Полученную суспензию центрифугируйте при 11000 g.*
2. Поместите 60 мкл надосадочной жидкости в микропробирку (с набором не поставляется)	
3. Добавьте к образцу 180 мкл Лизирующего буфера1 и 25 мкл Протеиназы К. Тщательно встряхните.	*Если проводится определение нескольких образцов допускается предварительно смешать Лизирующий буфер 1 и Протеиназу К и сразу добавить к пробам.*
4. Инкубируйте при 56°С в течение 10 минут при постоянном перемешивании.	
5. Перемешайте на вортексе, центрифугируйте и поместите пробу в пробирку для образцов.	*Если проба содержит видимые частицы – центрифугируйте при 11000 g и надосадок переместите в пробирку для образцов.*
6. Добавьте 200 мкл Лизирующего буфера 2 в пробирку для образцов.	
7. Тщательно перемешайте на вортексе 10 – 15 сек	
8. Инкубируйте 10 минут при 70 °С.	
9. Перемешать на вортексе 10 – 15 сек	
10. Слегка центрифугируйте пробирку для образцов на вортексе чтобы удалить капли с крышки	*Кратковременно*
11. Добавьте 10 мкл ВКО	
12. Добавьте 210 мкл этанола (96-100 %) и Перемешать на вортексе 10 – 15 сек	*После добавления этанола может образоваться преципитат. Это не повлияет на полноту извлечение ДНК. Проследите, чтобы весь преципитат был перенесен на колонку*
13. Осторожно загрузите лизат на колонку фильтр, поместите её в пробирку для отбора НК и закройте крышку.	*Центрифугировать 1 мин при 11,000 x g*
14. Поместите колонку фильтр в новую пробирку для отбора НК (2 мл, прилагается) и избавитесь от пробирки с	

жидкостью, полученной на предыдущем этапе.	
15. Добавьте 500 мкл Промывочного буфера 1 на колонку для фильтрации.	*Центрифугировать 1 мин при 11,000 x g*
16. Избавитесь от проточной жидкости, полученной на предыдущем этапе. Верните колонку фильтр в пробирку для отбора НК.	
17. Добавьте 600 мкл Промывочного буфера2 на колонку для фильтрации.	*Центрифугировать 1 мин при 11,000 x g*
18. Избавитесь от проточной жидкости, полученной на предыдущем этапе. Верните колонку фильтр в пробирку для отбора НК.	
19. Удалите оставшееся количество этанола.	*Центрифугировать 3 мин при 11,000 x g*
20. Поместите колонку для фильтрации в пробирку для элюирования (1.5 мл не прилагаются к набору) и избавитесь от пробирки с жидкостью, полученной до этого.	
21. Добавьте 60 мкл Элюента и инкубируйте 1-2 мин при комнатной температуре	*Центрифугировать 1 мин при 11,000 x g*
22. Выделенное ДНК может храниться в условиях глубокой заморозки (–20…–80°С) в течение нескольких месяцев; допускается хранение при +2…+8 в течение суток.	

Выделение ДНК из образца ткани, мясной продукции, цельной крови сорбентным методом

Осуществляют с помощью комплекта для выделения нуклеиновых кислот (НК), входящего в состав тест-системы.

Таблица 2.3: Компоненты комплекта.

Наименование	Количество на 50 проб мл
Лизирующий раствор	7,5
Сорбент	1,0
Промывочный раствор №1	10,0
Промывочный раствор №2	10,0
Промывочный раствор №3	10,0
Элюирующий раствор	5,0

Необходимые реагенты и оборудование

- Одноразовые наконечники (рекомендуются наконечники с фильтром и лабораторный пластик RNA-se/DNA-se Free)
- Пипетки
- Центрифуга для маленьких пробирок высокоскоростная
- Вортекс
- Термостат для инкубации при 70 °С
- Средства личной защиты (например, лабораторный халат, перчатки, очки)
- Аспиратор медицинский

Хранение и стабильность

Комплект реагентов для выделения следует хранить при температуре 2–8°С в течение всего срока годности. После вскрытия упаковки лизирующий раствор и промывочный раствор №1 следует хранить в темном месте. Срок годности комплекта – 6 месяцев.

Выделение вирусной ДНК

Шаг
1. Образцы патматериала (селезенка, почки, легкие, лимфоузлы и др.) должны быть измельчены специальными механическими гомогенизаторами (25 мг ткани поместить в пробирку Эппендорф добавить 250 мкл ФБР или физраствора и гомогенизировать). Полученную суспензию центрифугируйте при 11000 g. 25 мг пищевого продукта свиного происхождения (колбаса, сосиски) поместить в пробирку Эппендорф, добавить 250 мкл воды очищенной, встряхнуть на вортексе и прогреть в течение 5 мин при 65°С. Центрифугируйте при 11000 g.
2. Внесите по 60 мкл подготовленного биоматериала в пробирки для исследуемых образцов. В пробирку «К-» биоматериал не вносится.
3. В пробирку, маркированную «К-», внесите 60 мкл физиологического раствора стерильного.
4. В каждую пробирку внесите по 10 мкл ВКО.
5. Приготовьте смесь лизирующего раствора с сорбентом. Смешайте в отдельной пробирке: 150 x (N+1) мкл лизирующего раствора, 20 x (N+1) предварительно ресуспендированного сорбента,

	где N + 1 – количество анализируемых образцов с учётом «K-» (N) с запасом на 1 образец.
6.	Добавьте в каждую пробирку по 170 мкл полученной смеси.
7.	Плотно закройте крышки пробирок, встряхните на вортексе в течение 3–5 сек.
8.	Термостатируйте пробирки в течение 15 мин при 50°C.
9.	Центрифугируйте пробирки при 11000 g в течение 1 мин.
10.	Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки)
11.	Добавьте к осадку 200 мкл промывочного раствора №1 и встряхните пробирки на вортексе в течение 3–5 сек.
12.	Центрифугируйте пробирки при 11000 g в течение 1 мин.
13.	Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).
14.	Добавьте к осадку 200 мкл промывочного раствора №2 и встряхните пробирки на вортексе в течение 3–5 сек.
15.	Центрифугируйте пробирки при 11000 g в течение 1 мин.
16.	Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).
17.	Добавьте к осадку 200 мкл промывочного раствора №3 и встряхните пробирки на вортексе в течение 3–5 сек.
18.	Центрифугируйте пробирки при 11000 g в течение 1 мин.
19.	Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).
20.	Откройте крышки пробирок и высушите осадок при 50°C в течение 5 мин.
21.	Добавьте к осадку 60 мкл элюирующего раствора и встряхните пробирки на вортексе в течение 5–10 сек.
22.	Прогрейте пробирки при 50°C в течение 5 мин.
23.	Центрифугируйте пробирки при 11000 g в течение 1 мин. Если образец предполагается хранить более 7 суток, перенесите надосадочную жидкость в новую пробирку.

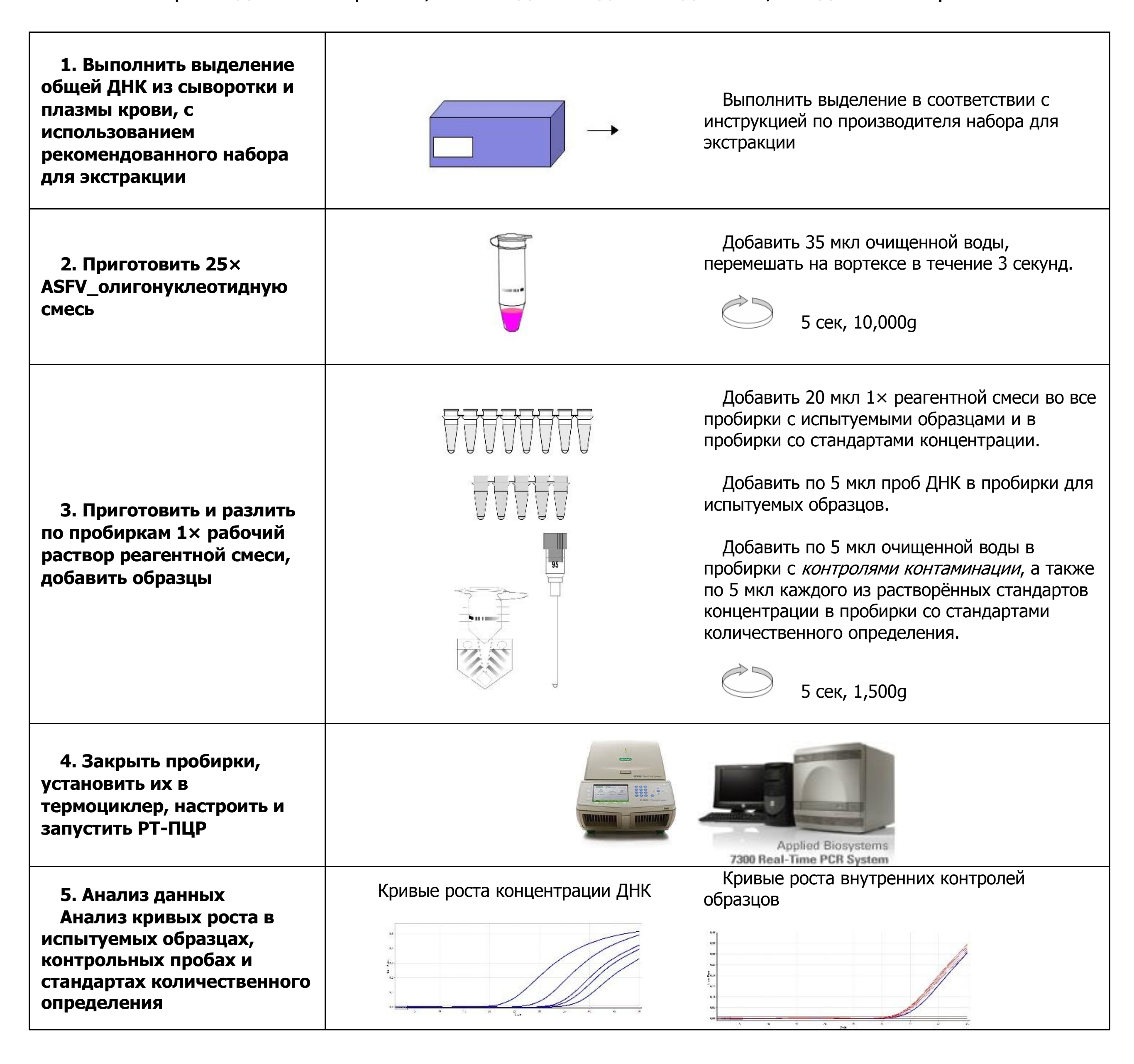
Надосадочная жидкость, содержащая выделенную ДНК, готова к внесению в реакционную смесь для ПЦР–амплификации.

Полученный препарат ДНК можно хранить до 5 суток при температуре 2–8°C. Более 5 суток препарат ДНК следует хранить при температуре минус 20°C. Срок хранения — до 6 месяцев

Принцип действия набора для определения ASFV

Набор для определения *ASFV* – это тест-система, основанная на полимеразной цепной реакции в реальном времени (Real-Time PCR). Набор разработан для качественной детекции транскриптов *ASFV*. Стандарт качественного определения состоит из синтетической ДНК *ASFV*, которые должны амплифицироваться вместе с анализируемыми образцами.

Рисунок 1: набор для определения *ASFV*: краткий обзор протокола эксперимента.
Реагенты и расходные материалы, необходимые для стадий 1-2, входят в набор.



1. Выполнить выделение общей ДНК из сыворотки и плазмы крови, с использованием рекомендованного набора для экстракции	Выполнить выделение в соответствии с инструкцией по производителя набора для экстракции
2. Приготовить 25× ASFV_олигонуклеотидную смесь	Добавить 35 мкл очищенной воды, перемешать на вортексе в течение 3 секунд. 5 сек, 10,000g
3. Приготовить и разлить по пробиркам 1× рабочий раствор реагентной смеси, добавить образцы	Добавить 20 мкл 1× реагентной смеси во все пробирки с испытуемыми образцами и в пробирки со стандартами концентрации. Добавить по 5 мкл проб ДНК в пробирки для испытуемых образцов. Добавить по 5 мкл очищенной воды в пробирки с *контролями контаминации*, а также по 5 мкл каждого из растворённых стандартов концентрации в пробирки со стандартами количественного определения. 5 сек, 1,500g
4. Закрыть пробирки, установить их в термоциклер, настроить и запустить РТ-ПЦР	Applied Biosystems 7300 Real-Time PCR System
5. Анализ данных **Анализ кривых роста в испытуемых образцах, контрольных пробах и стандартах количественного определения**	Кривые роста концентрации ДНК Кривые роста внутренних контролей образцов

Универсальный протокол для устройств, поддерживающих детекцию FAM или FAM/HEX флуоресценции

Описанные примеры приведены для Real-Time PCR системы RotorGene и BioRad CFX96^{TM}



Важно! *Включайте в состав проб, по крайней мере, один положительный контроль ДНК ASFV, отрицательный контроль Вода для ПЦР-исследований, а также контроли контаминации реагентов, содержащие вместо исследуемых образцов воду для PCR-исследований*. Контрольный образец входит в состав данного набора. Отрицательные контроли и контроли контаминации, в которых произошла амплификация, указывают на контаминацию проб экзогенным генетическим материалом. В этом случае необходимо повторить РТ-ПЦР, используя свежеприготовленные реагенты, и образцы необходимо исследовать как минимум два (лучше – три) раза подряд.

В целях сохранения полной активности Taq-полимеразы все операции по дозированию и смешиванию реактивов необходимо производить во льду либо на специальной охлаждаемой поверхности!!

Таблица 4. Этапы работы

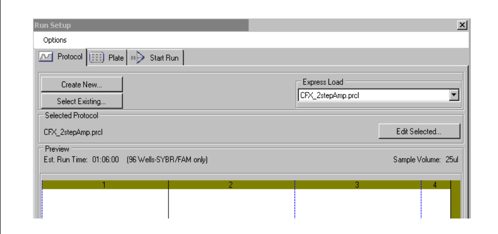
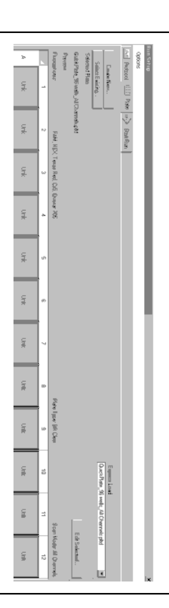
Этап работы	Замечания и комментарии
Приготовление стандартов	
1. Разморозить стандарт качественного определения до комнатной температуры. Встряхните и центрифугируйте на центрифуге-вортекс.	При работе с испытуемыми образцами и стандартными пробами всегда одевайте перчатки.
Проведение ПЦР	
2. *Приготовление 25× концентрата смеси реагентов из лиофилизированного ASFV-олигонуклеотидного микста.* Центрифугируйте пробирки с олиго микстом на центрифуге-вортекс перед тем, как их открыть, чтобы порошок лиофилизированных олигонуклеотидов гарантированно переместился со стенок пробирок на дно и не разлетелся при открывании пробирки. Добавьте 35 мкл очищенной воды во флакон, содержащий лиофилизированный олиго микст. Закройте флакон и перемешайте на вортексе в течение 3 секунд, затем центрифугируйте в течение 5 секунд на центрифуге-вортекс.	Растворённая смесь реагентов может храниться при +2-8°C в защищённом от света месте в течение минимум 3 месяцев (не замораживать!).
3. Приготовление рабочего раствора основной реагентной смеси (**Таблица 5**). Подсчёт общего количества проб, подвергаемых ПЦР (испытуемые пробы + все контрольные образцы). Перемешайте подготовленную смесь на вортексе в течение 3 секунд, затем центрифугируйте в течение 5 секунд на центрифуге-вортекс.	Мы рекомендуем добавлять в расчёт 1-2 дополнительных пробы, чтобы компенсировать неточности при дозировании и потери при пипетировании. Все вышеперечисленные манипуляции производятся **на льду!**

Таблица 5: Перечень и объёмы реагентов для приготовления рабочего раствора основной реагентной смеси. Готовую смесь перемешать на вортексе и осадить капли с крышки на центрифуге-вортекс.

Реагент	**Без ROX**	**Высокая концентрация ROX (1×)[1)]**	**Низкая концентрация ROX (0.1×)[2)]**	
	мкл в 1 пробе	**мкл в 1 пробе**	**мкл в 1 пробе**	**Окончательная концентрация**
Вода для PCR-исследований	16.1	15.6	16.05	–
Буферная смесь2	2.5	2.5	2.5	1×
ASFV-олигонуклеотидный микст	1.0	1.0	1.0	1×
Taq-полимераза (5 Ед/мкл)	0.4	0.4	0.4	2.0 Ед в 1 пробе
50× ROX	–	0.5	0.05[3)]	1× / 0.1×

[1)] Для работы с семейством термоциклеров ABI PRISM® 7000/ 7300/7700 (Thermo Fisher Scientific)
[2)] Для работы с семейством термоциклеров 7500 Fast Real Time PCR System, StepOne® (Thermo Fisher Scientific)
[3)] Если количество проб для PCR невелико – предварительно растворите 50× концентрат ROX водой для PCR-исследований в соотношении 1:10, затем добавьте по 0,5 мкл полученного раствора в каждую пробу. В этом случае в каждую пробу необходимо добавить по 18.6 мкл воды для PCR-исследований.

Этап работы	**Замечания и комментарии**
4. Приготовьте пробирки в количестве, достаточном для амплификации контролей (ОКО, К+, К-) и испытуемых образцов, поместите их в охлаждаемый штатив.	⚠ При работе с образцами и стандартами всегда одевайте перчатки.
5. Добавьте 20 мкл рабочего раствора основной реагентной смеси (**см. табл. 5**) во все пробы.	
6. Перенесите по 5 мкл воды для PCR-исследований в пробирки с отрицательными контролями не содержащими образцов (т.н. *контроли контаминации – К-*) и 5 мкл стандарта (К+) в пробирку с положительным контролем.	⚠ Итоговый объём реакционной смеси не должен превышать 25 мкл.
7. Добавьте 5 мкл проб ДНК в соответствующие пробирки с испытуемыми образцами.	⚠ Итоговый объём реакционной смеси не должен превышать 25 мкл.
8. Закройте пробирки с пробами соответствующими крышечками, стрипами крышечек либо оптической плёнкой.	
9. Сбросьте жидкость на дно пробирок центрифугированием на центрифуге-вортекс в течение 5 секунд.	
10. Аккуратно поместите подготовленные пробирки в термоциклер, закройте крышку.	

BIO RAD CFX 96	
11. Запустите настройку протокола ПЦР: a. Включите инструмент нажатием на кнопку питания, затем запустите программу CFX Manager. b. Для создания нового протокола выберите «Create New» («создать новый»): откроется редактор протоколов; в противном случае выберите «Select Existing» («выбрать существующий»): запустится файловый менеджер, где можно будет выбрать для использования или редактирования существующий файл протокола. Можно также использовать выпадающее меню «Express Load» («экспресс-загрузка»).	 ⚠ Если вы используете ПЦР-оборудование, отличающееся от описываемого, строго следуйте рекомендациям производителя, принимая во внимание отличия в настройках своего инструмента.
c. Редактор протокола Создайте новый протокол или запустите существующий**.** Выберите любой этап протокола на графическом либо текстовом экране. Нажмите «Add plate read to Step» («добавить к этапу считывание с планшеты»), чтобы задать момент считывания данных флуоресценции. Задайте момент считывания данных с планшеты **на этапе отжига/ элонгации** при 59°С. Кликните на количество повторов (этап «GOTO»), установите количество циклов в петле равным 45.	
12. Перейдите на вкладку «Setup Plate» («настройка планшеты»). a. Нажмите «Create New» («создать новый»), чтобы открыть редактор планшет и создать новую схему эксперимента. Иначе, кликните на «Select Existing» («выбрать существующий»), чтобы запустить файловый менеджер и выбрать существующий файл со схемой эксперимента для использования или редактирования. b. Используя выпадающее меню «Express Load» («экспресс-загрузка»), выберите файл по умолчанию «QuickPlate_96wells_AllChannels.pltd». c. Перейдите на вкладку «Start Run» («запустить»), чтобы запустить ПЦР с имеющейся схемой эксперимента.	

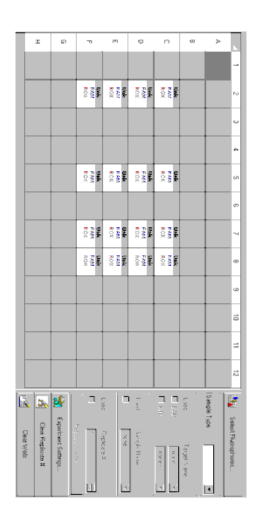
13. Редактор планшет: используется для создания новой схемы эксперимента или редактирования существующей. a. Используя панель инструментов редактора планшет, укажите режим сбора данных, который планируете использовать. b. Кликните на кнопку «Select Fluorophores» («выбор флюорохромов») и укажите, что в данном эксперименте нужно использовать FAM (вирусная ДНК) и HEX (внутренний контроль). c. На схеме планшеты укажите используемые ячейки. d. выберите в выпадающем меню «Sample Type» («тип проб»). e. Установите необходимый флажок (или несколько), чтобы задать красители, соответствующие выбранным ячейкам. f. Впечатайте в каждую ячейку название соответствующей пробы, нажмите «Enter».	
Rotor-Gene	
14. Создайте новый протокол или запустите существующий. a. Нажать кнопку New в меню программы b. Выбрать тип ротора. Поставить отметку No Domned 0.2 ml c. Выбрать объем реакционной смеси 25 мкл d. Задать параметры амплификации в соответствии с **Таблицой 6**. e. Измерение флуоресценции по каналам FAM (Зеленый) и HEX (Желтый) проводят при 59°C	
15. Настройку каналов детекции провести согласно таблице 7	
16. Запустить амплификацию кнопкой Start run	

Таблица 6: Условия амплификации для количественного определения ДНК *ASFV (универсальная для всех термоциклеров)*

Этап	Температура	Время	Число повторов
Активация Taq-полимеразы	95°C	4:00 мин	1
Плавление (денатурация)	95°C	5 с	45
Отжиг/элонгация/детекция флуоресцентного сигнала (FAM/HEX)	59°C	30 с	

Общее время, необходимое для завершения РТ-ПЦР по данному протоколу – приблизительно 2 час 00 минут.

Таблица 7: Настройка канала детекции, а также других параметров оборудования

РТ-ПЦР-термоциклер	Канал детекции	Прочие параметры оборудования
No-ROX		
Rotor-Gene™ 3000/6000, Rotor-Gene™ Q (Qiagen)	Green (Зелёный) Yellow (Желтый)	Rotor-Gene 3000: Dynamic tube: Yes Slope correction: Yes Ignore first: 4 No template control threshold: 5% Perform Calibration/Optimisation Before 1th Acquisition – Min Reading 5Fl – Max Reading 10Fl Threshold modus: manual ASFV: 0.01-0.04, на экспоненциальной части амплификационной кривой, выше фоновых значений, стремясь к минимальному значению
		Rotor-Gene 6000/Q: Dynamic tube: Yes Slope correction: Yes Ignore first: 1 No template control threshold: 5% Perform Calibration/Optimisation Before 1th Acquisition – Min Reading 5Fl – Max Reading 10Fl Threshold modus: manual ASFV: 0.01-0.04, на экспоненциальной части амплификационной кривой, выше фоновых значений, стремясь к минимальному значению
CFX96TM; iCycler IQ™; IQ5, MiniOpticon™, CFX Connect™ Real-Time PCR Detection System (Bio-Rad)	FAM	CFX96: необходимо вручную установить пороговое значение в 50-250 относительных единиц флуоресценции (RFU).
SmartCycler II® (Cepheid)	FAM (канал 1)	нет
Mx3000P, Mx3005P (Agilent/Stratagene)	FAM/SYBR	Проверьте, чтобы не был установлен референсный краситель
LightCycler® 480II, LightCycler® 96 (Roche)	FAM	нет
Mastercycler® ep realplex (Eppendorf)	FAM	нет
Высокая/низкая концентрация пассивного ROX		
7500 Fast Real Time PCR System	FAM	Выберите «*auto baseline*» («автоматический базовый уровень»), вручную установите пороговое значение ≥0.02
ABI PRISM® 7000/7300/7700 Sequence Detection System, StepOne® (Thermo Fisher Scientific)	FAM	Выберите «*auto baseline*» («автоматический базовый уровень»), вручную установите пороговое значение >0.05

Анализ данных

Каждый цикл амплификации ДНК приводит к генерации флуоресцентного сигнала, измеряемого в «Зелёном» (**FAM**) канале для **мишени** и «Желтом» (**HEX**) для **внутреннего контрол**я, что приводит к формированию сигмовидной кривой роста (в логарифмической шкале). Следует выполнять анализ данных в соответствии с рекомендациями производителя оборудования (см., например, руководство по использованию термоциклера Rotor-Gene 6000/Q или CFX96™ Real Time PCR Detection System, Bio-Rad) с использованием соответствующего программного обеспечения и учитывая рекомендации, приведённые в **Таблице 7** (а также нижеследующие замечания). Определение ДНК *ASFV* в испытуемых пробах основано на значениях C_T *(порогового цикла)* кривых роста, полученных при амплификации испытуемых образцов ДНК.

1. При значении C_T по каналу FAM менее или равный 40, и значении C_T по каналу HEX менее 40 образец содержит фрагменты генома ASFV (положительный).
2. При значении C_T по каналу FAM более 40 или его отсутствии (N/A) и значении C_T по каналу HEX менее 40 образец не содержит фрагменты генома ASFV (отрицательный).
3. При значении C_T по каналу FAM менее или равный 20, и отсутствии значения C_T по каналу HEX (N/A) образец содержит фрагменты генома ASFV (положительный).
4. При значении C_T по каналу FAM более 20, и отсутствии значения C_T по каналу HEX (N/A) необходимо повторить исследование образца, в том числе в двух десятикратных разведениях. Если при этом в одной из проб обнаружатся сигмоидные кривые по каналу FAM и HEX – образец содержит фрагменты генома ASFV (положительный).
5. При отсутствии значения C_T по каналу FAM (N/A), и отсутствии значения C_T по каналу HEX (N/A) результат считается невалидным. Необходимо повторить исследование образца (n = 2), включая этап экстракции.
6. Для достоверной интерпретации результатов учитывать кинетические кривые накопления флуоресценции только сигмоидальной формы.

Интерпретация	Значение C_T по каналу FAM	Значение C_T по каналу HEX
Положительный (+)	≤ 40	≤40
Отрицательный (–)	N/A	≤40
Положительный (+)	≤ 20	N/A
Повторить исследование, включая разведения	≥20	N/A
Повторить исследование (n=2)	N/A	N/A

⚠ *В случае появления сомнительных положительных результатов возможна контаминация инструментов и рабочего места – следует провести деконтаминационные мероприятия в лаборатории.*

⚠ *Установленный пороговый уровень детекции может существенно повлиять на значения C_T. Устанавливайте величины пороговых уровней в соответствии с рекомендациями из **Таблицы 7**.*

Решение проблем

Таблица 8: Возможные причины ошибок и как их устранить

Проблема	Возможная причина проблемы	Решение
Проблемы, связанные с подготовкой образцов		
Недостаточная степень чистоты экстрагированной ДНК или синтезированной ДНК	Контаминация образцов РНК/ДНК солями белков, углеводами и прочей органикой, ингибирующей ПЦР	Избегать методов фенольной и/или других методов экстракции, использовать только прилагаемый набор для экстракции нуклеиновых кислот.
Проблемы, связанные с пипетированием		
Получен флуоресцентный сигнал из проб, не содержащих ДНК *ASFV*, и/или из контролей контаминации реагентов	Контаминация негативных проб ампликонами *ASFV*	Повторить экстракцию и/или ПЦР с новыми реагентами; провести деконтаминацию инструментов и рабочего места.
Итоговый объём реакционной смеси отличается от 25 мкл	Ошибки пипетирования, например, пропуск либо повторная заливка ячеек	Используйте мультиканальные пипетки, автоматизированное пипетирование, либо развивайте внимание и концентрацию.

Проблемы, связанные с амплификацией		
Необычно большие значения C_T стандарта и/или завышенная концентрация ДНК *ASFV* в испытуемых образцах	Неверный протокол амплификации	Проверьте настройки оборудования, следуйте инструкциям из руководства по пользованию прибором.
	Нарушение условий и/или сроков хранения реагентов	Проверьте условия хранения и срок годности.
	Распад определяемой ДНК	Используйте расходные материалы и реагенты, не содержащие нуклеаз; немедленно после синтеза помещайте образцы ДНК на лёд.
Не сигмовидная форма кривых роста стандартов концентрации и испытуемых образцов	Частое размораживание/оттаивание либо неправильное хранение смеси растворённых реагентов	Прочтите инструкцию, проверьте условия хранения, приготовьте свежую реагентную смесь.
	Условия хранения не соответствуют рекомендуемым, истёк срок хранения набора	Проверьте условия хранения и срок годности.
Отсутствует флуоресцентный сигнал	Измерение флуоресцентного сигнала отключено; камера неправильно установлена	Проверьте настройки оборудования.
	Выбран неправильный канал записи флуоресцентного сигнала	Для определения ДНК *ASFV* выберите канал FAM. Для определения внутреннего контроля выберите канал HEX.
	Неверный протокол амплификации	Проверьте настройки оборудования.
	Нарушение условий и/или сроков хранения набора	Проверьте условия хранения и срок годности.

Различный вид амплификации ДНК *ASFV* в испытуемых образцах, непараллельный рост кривых в экспоненциальной фазе реакции	Избыток ингибиторов ПЦР в пробе	Используйте рекомендованный набор для экстракции, точно следуйте инструкциям производителя. Разведение ДНК перед анализом может снизить содержание ингибиторов в образце (см. протокол, этап 7).
	Неправильно собранный материал (н-р, гепаринизированная кровь)	Используйте правильно собранные образцы.
Регистрируется низкий уровень флуоресцентного сигнала в ходе амплификации определяемой ДНК	Загрязнение оптических линз	См. раздел «уход» инструкции по использованию соответствующего термоциклера; если позволяет конструкция – раз в месяц протирать линзы, используя абсолютный этиловый спирт и ватные палочки.
	Загрязнение системы охлаждения и/или матицы оптического сенсора	См. раздел «уход» инструкции по использованию соответствующего термоциклера; можно также заполнить каждую ячейку сенсора изопропанолом, инкубировать 10 минут при 50°C, удалить изопропанол и промыть бидистиллированной водой.
	Испарение из ячейки в ходе ПЦР	Крышки планшет, пробирок, стрипов должны быть максимально эффективными.
	В случае если инструмент требует использования пассивного красителя ROX – использование неправильной концентрации ROX	Используйте раствор ROX оптимальной концентрации, поставляемый с данным набором; убедитесь, что используете «AR» версию набора.